GENERAL LIBRARY
AUG 27 1918
UNIV. OF MICH.
SCIENCE LIBRARY

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

VOL. XXXV AUGUST, 1918 NO. 2

CONTENTS

PAGE

COWDRY, N. H. *The Cytology of the Myxomycetes with Special Reference to Mitochondria* .. 71

SHINJI, GEORGE O. *A Contribution to the Physiology of Wing Development in Aphids* 95

FAUST, ERNEST CARROLL. *Eye-Spots in Digenea* 117

PUBLISHED MONTHLY BY THE
MARINE BIOLOGICAL LABORATORY

PRINTED AND ISSUED BY
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

AGENT FOR GREAT BRITAIN
WILLIAM WESLEY & SON
28 Essex Street, Strand, London, W. C.

Single Numbers, 75 Cents. Per Volume (6 numbers), $3.00

Entered October 10, 1902, at Lancaster, Pa., as second-class matter under Act of Congress of July 16, 1894.

Editorial Staff

E. G. Conklin—*Princeton University.*

Jacques Loeb—*The Rockefeller Institute for Medical Research*

George T. Moore—*The Missouri Botanic Garden.*

T. H. Morgan—*Columbia University.*

W. M. Wheeler—*Harvard University.*

E. B. Wilson—*Columbia University.*

Managing Editor

Frank R. Lillie—*The University of Chicago.*

All communications and manuscripts should be sent to the Managing Editor, the University of Chicago, Sept. 15th to June 15th, or Woods Hole, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, 41 North Queen Street, Lancaster, Pa

Vol. XXXV. *August, 1918.* *No. 2*

BIOLOGICAL BULLETIN

THE CYTOLOGY OF THE MYXOMYCETES WITH SPECIAL REFERENCE TO MITOCHONDRIA.[1]

N. H. COWDRY,

ANATOMICAL LABORATORY, JOHNS HOPKINS UNIVERSITY.

The Myxomycetes, or Slime moulds, constitute a most interesting group of organisms since they are at once so primitive and so specialized and partake of the distinctive properties of both animals and plants. They stand as a sort of link between the two kingdoms. In the plasmodial phase of their existence, for example, they look like gigantic amœbæ, crawl from place to place, exhibit typical protoplasmic streaming and actively phagocytize foreign particles. In the reproductive phase, on the other hand, they form brilliantly colored fungous-like masses strongly suggestive of plants. It is not surprising, therefore, that they have attracted so much interest among botanists and zoölogists alike. Their general form and nuclear structure has been carefully worked up, but no attempt has been made to extend to them the recent work on mitochondria. This is all the more surprising, because the unique properties of these organisms would lead one to suppose that a careful study of mitochondria in them might yield valuable information bearing upon the Myxomycetes themselves, as well as upon the vexed problem of the general functional significance of mitochondria.

MATERIAL AND METHODS.

The following species of Myxomycetes have been studied:

Arcyria denudata, *Fuligo septica*,
Badhamia ———, *Hemitrichia vesparium*,

[1] Contributions from the Anatomical Laboratory, Peking Union Medical College, No. 2.

Ceratiomyxa ———,	*Hemitrichia clavata*,
Cribraria ———,	*Lycogala epidendrum*,
Enteridium rozeanum,	*Stemonitis* ———.

They were collected during June, July and August near South Harpswell, Me., and during September, October and November in the vicinity of Baltimore, Md. At South Harpswell, the Director, Dr. Kingsley, very kindly placed the resources of the biological laboratory at my disposal and I wish to thank him for his courtesy.

Portions of the plasmodia were collected, shortly before sporangium formation, on the surfaces of leaves, mosses and damp logs. Immature sporangia were found showing well all the stages between the undifferentiated plasmodium and the young spores. These were placed immediately in the fixative. Smears of the plasmodium were treated in the same manner with results which were only confirmatory, but by no means so distinct or satisfactory.

Fixation:

1. Pieces not larger than 4 cubic millimeters were placed in the following mixture:

Commercial formalin . . . . . 5 c.c.
(A mixture of formaldehyde, water and methyl alcohol should not be used.)
3 per cent. potassium bichromate . . . . . 20 c.c.
4 to 5 days, changing daily.

2. 3 per cent. potassium bichromate, changing every second day, 7 to 8 days.

3. Wash in running water 24 hours.

This is the ordinary Regaud ('10, p. 296) IVB fixative which can be modified with very excellent results, in some cases, by diluting the fixative with an equal volume of water, applying it for 2 to 4 days only and the bichromate for 3 to 5 days as recommended by Sapehin ('15, p. 321).

Another very good fixative is Regaud IVA:

1. Formalin . . . . . 10 to 50 c.c.
Water . . . . . 50 c.c.
for 1 to 5 days.

2. 3 per cent. potassium bichromate, 3 to 4 weeks.

3. Wash in running water, 1 day.

I have found that formalin in 5 per cent. or 10 per cent. solution preserves the normal form of mitochondria better than higher concentrations. The subsequent long mordanting in potassium bichromate may sometimes be omitted with equally satisfactory results.

Other mitochondrial fixatives have been devised containing chromic, picric or osmic acid in varying concentrations. These are the Benda, Champy and Regaud II and III which give very good results, but mitochondria are, I believe, more constant in their response to the Regaud mixtures given above.

Sections should be cut about 3 or 4 μ in thickness.

Staining:

The Heidenhain Iron Hematoxylin Method:

1. 5 per cent. iron alum, 24 hours.
2. Wash in water, 5 minutes or less.
3. 1 per cent. hematoxylin, 24 hours.
   (Made by dissolving 10 gm. hematoxylin in 100 c.c. of absolute alcohol. This should be kept until ripe, when 10 c.c. of the mixture should be added to 90 c.c. of distilled water.)
4. Wash in water, 5 minutes.
5. Differentiate in 2 per cent. iron alum under the microscope.
6. Wash in water at least 1 hour.
7. Pass through 50 per cent., 70 per cent. and 90 per cent. to absolute alcohol, clear in xylol and mount in balsam.

The Bensley Method (E. V. Cowdry, '16b, p. 30):

1. 1 per cent. potassium permanganate, 30 seconds.
2. 5 per cent. oxalic acid, 30 seconds.
3. Rinse in water.
4. Stain in Altmann's anilin fuchsin (anilin water 100 c.c., acid fuchsin 20 gm.), heat once until vapor arises, 6 minutes.
5. Rinse quickly in distilled water.
6. Differentiate in 1 per cent. aqueous solution of methyl green or toluidin blue very quickly.

7. Drain and dehydrate quickly with absolute alcohol.
8. Clear in xylol and mount in balsam.

The Altmann Method:

1. Stain on slide with Altmann's anilin fuchsin, heating once until vapor arises, 6 minutes.
2. Wash quickly in water.
3. Differentiate in alcoholic solution of picric acid (made by mixing one part of a saturated alcoholic solution with two parts of distilled water) until the sections assume a yellowish-pink color.
4. Dehydrate very quickly in absolute alcohol, clear in xylol and mount in balsam.

The Benda Method (*'01, p. 155*):

1. 4 per cent. iron alum, 24 hours.
2. Wash in water, 2 or 3 minutes.
3. Sulphalizarinate of soda, 24 hours. (Made by adding a saturated alcoholic solution of sulphalizarinate of soda drop by drop to distilled water until an amber color is obtained.)
4. Dry excess with blotting paper.
5. Cover with a solution of crystal violet, and warm until production of vapor begins, 5 minutes.

(Made by mixing

1 vol. saturated solution of crystal violet in 70 per cent. alcohol.

1 vol. acid alcohol.

2 vol. anilin water.)

6. Dry off excess with blotting paper.
7. Differentiate with 30 per cent. acetic about 3 minutes.
8. Dehydrate in absolute alcohol quickly.
9. Pass through xylol to balsam.

Observations.

The chief stages in the complicated life history may first be mentioned.

The vegetative phases or plasmodia are found in damp locations, on the surface of logs, fallen leaves and debris. They are

slippery, slimy masses, sometimes nearly a foot in diameter. The color is often very brilliant. For instance, in *Ceratiomyxa* it is white, *Fuligo septica* yellow, *Lycogala epidendrum* light red and *Hemitrichia vesparium* a beautiful dark crimson. But the color is of no generic significance because different species of the same genus exhibit great variability. It is to be noted that they never, under any circumstances, contain chlorophyll, and in this respect they differ sharply from all plants which are not saprophytic and closely approximate to animals. Plasmodia are naked masses of protoplasm containing abundant nuclei, but destitute of cell walls, thus resembling in many ways the syncytia of higher animals and large multinucleate giant cells. They apparently possess all the properties of amœbæ, especially the power of amœboid movement and of being actively phagocytic. They engulf bacteria and foreign particles in much the same way as the so-called macrophages which have been brought into prominence lately in mammals through the use of vital dyes.

In some cases, when the conditions become unfavorable, the nuclei tend to clump together into larger or smaller masses which encapsulate and desiccate. This resting stage, called the sclerotium, may persist for some time. On the resumption of favorable conditions the envelopes are dissolved and the plasmodia reformed.

The reproductive stages are just as remarkable. The plasmodium first migrates to the upper surface of the log or stump or other object, as the case may be, where it will be exposed to more light. It then undergoes great and varied changes in different species. It may form a cushion-like mass, an æthalium, as in *Fuligo;* a flat vermicular aggregation called a plasmodiocarp; or a number of separate sporangia as in *Hemitrichia clavata.* The sporangia may be either sessile or elevated on pedicels. They are surrounded by a definite envelope, termed the sporangium wall, which may even be double. The sporangia contain spores of many hues and varied sculpture and in most cases a capillitium. The capillitium, which is composed of tubes or of threads, generally arranged in the form of a network, is sometimes supportive and may be, at the same time, concerned with the dispersal of the spores. The sporangium wall, the spore wall and the capillitium are all differentiations of the plasmodium.

On germination the protoplasmic content of the spore escapes. It soon becomes actively motile, develops a flagellum and food vacuole and reminds one forcibly of the flagellates. The flagellum is withdrawn and the organism either goes into a brief resting stage (microcyst) or multiplies freely by fission. In the case of multiplication by fission there is a karyokinetic figure and distinctive chromosomes may be seen. Finally these swarm cells clump together and fuse to form another plasmodium.

The granulations about to be described are identified as mitochondria on the basis of the following observations:

1. Their morphology is identical with that of mitochondria in the higher forms of both plants and animals. While they are for the most part spherical, rod-shaped forms do occur.

2. Their distribution is also characteristic. They are generally single but are often arranged in rows like streptococci, or in clumps. They seem to be rather more abundant near the nuclei and about the circumference of the vacuoles.

3. The janus-green reaction is exhibited beautifully by the mitochondria when the contents of the adult spores are crushed out in janus-green solution.

4. They are easily fixed by Regaud's mixtures, as indicated above.

5. They may be stained by the standard mitochondrial methods including the iron-hematoxylin method, the Altmann method, fuchsin and methyl green, and the Benda method.

I have been able to discover no descriptions relating to them in the literature except possibly that of Harper ('00, p. 251, fig. 18), who made a study of cell and nuclear division in *Fuligo varians*, and found certain granules, in a single spore cell only, which he refers to as "granules of reserve material," in a preparation fixed in Flemming's weak fluid and stained with safranin, gentian violet and orange. These granulations present the same morphology as mitochondria, but in the total absence of detailed information, there is no means of ascertaining their nature.

In *Enteridium rozeanum*, we find comparatively large areas of protoplasm in which no differentiations of any sort can be distinguished. There are no nuclei, no mitochondria and but few vacuoles (Fig. 1). Such extensive areas of protoplasm without

visible organization, are, I believe, without counterpart in any other living organisms, outside the Myxomycetes. We must not commit the usual error, however, of calling this protoplasm homogeneous, because homogeneous protoplasm would be incapable of any vital manifestations or of activity of any sort. They stain light gray with iron hematoxylin, green with fuchsin methyl green, yellow with the Altmann method, and a dull pink by the Benda method. Around their margins groups of nuclei with a few mitochondria may be seen and a greater tendency toward vacuolation is noted.

In other parts of the plasmodium the mitochondria are fairly abundant (Fig. 5). They are for the most part spherical and of quite uniform size, varying between 0.25 and 0.5μ in diameter. The absence of the really tiny mitochondria, to be seen occasionally in higher forms, should be noted. Since the mitochondria never exceed these limits, the possibility of plast formation may be definitely excluded, except perhaps along the margins of the capillitial vacuoles. Rod-like mitochondria occur but they are quite rare. Filamentous, net-like and ring forms were never seen. The spherical mitochondria often clump together in pairs like diplococci or in linear series like streptococci. There can be no confusion with bacteria, however, because of their perfectly definite and characteristic reactions to fixatives and stains, which have already been mentioned. They are likewise spherical and look quite homogeneous. They stain quite darkly with iron hematoxylin and undergo definite modifications with the approach of spore formation, to be described subsequently. The ground substance is but faintly vacuolated and the nuclei are scattered irregularly, but fairly evenly.

Sometimes the vacuolation of the ground substance is much more marked, as is illustrated in Fig. 3. The vacuoles themselves are quite large and are usually, though not always, spherical. They often seem to run together. They contain for the most part a colorless liquid, never distinct spherules of protein as in the so-called "vacuoles de ségrégation" of Renaut and Dubreuil. Their walls are merely separation membranes and cannot be distinguished except in *Cribraria* where well formed and quite thick boundaries occur.

The mitochondria, which are also quite numerous, are frequently clumped about the periphery of the vacuoles, but they are never to be found within them, and this is another and very important distinction between mitochondria and bacteria which are phagocytized by Myxomycetes and are segregated and digested inside the vacuoles. The heaping up of mitochondria about the vacuoles may be merely a surface tension phenomenon. Their accumulation about the nuclei represented in Figs. 1, 3 and 4 may also be due in part to surface tension. It is important to bear in mind that the areas of clear protoplasm are, in their deeper parts, devoid of both mitochondria and vacuoles which become more numerous as we approach the nuclei, and that we may be dealing with nothing more than a heaping up of mitochondria in foci of more rapid metabolism.

Still more abundant mitochondria are shown in Figs. 4 and 6 but the vacuoles are fewer. Different parts of the organism are often separated by irregular, dense and homogeneous septæ, not related to capillitium threads, one of which is illustrated. It often happens that stretches of protoplasm separated in this way may exhibit a difference in the intensity of staining, the extent of vacuolation or in the number of mitochondria.

Fig. 2 of *Lycogala epidendrum* shows a portion of the active protoplasm of the plasmodium migrating upward through the interstices of the hypothallus to the æthalium where the spores will be formed. The nuclei are still spherical and of about the same size, but contain more distinct and prominent nucleoli. The mitochondria show little tendency toward perinuclear condensations. The protoplasm appears to be of rather open texture. The hypothallus presents an ill-defined fibrous appearance and is quite devoid of mitochondria and nuclei.

Spore Formation:

The early stages in spore formation in *Badhamia* are illustrated in Fig. 8. The nuclei, which were so conspicuous in the plasmodium, can no longer be seen with the aid of mitochondrial methods of staining. Their loss of affinity for iron hematoxylin and other basic dyes calls to mind the condition of affairs in oögenesis of certain animals where there is a temporary dis-

appearance of basophilic chromatic material. The ground substance of the protoplasm presents a sort of flocculent appearance, being denser in some regions than in others. The first indication that spore formation is taking place is to be seen in the clumping of the mitochondria. All the mitochondria are in the form of spherules of fairly uniform size. They stain with equal intensity with iron hematoxylin. The differences in shade illustrated are indicative of perspective only. Soon the little clumps of mitochondria become surrounded by a membrane, the first spore membrane, which stains only faintly with iron hematoxylin. Traces of nuclei can be seen in some of these early spores. Spore formation proceeds from a definite center so that it is possible to see many stages in a small area. Fig. 8 is taken through such an area, the early stages being above and the later stages below. In certain other Myxomycetes, however, spore formation is said to take place simultaneously throughout the whole sporangium.

Rather more advanced stages in *Fuligo septica* are shown in Figs. 7 and 9. Here all the spores are well formed and discrete and surrounded by membranes. The nuclei have reappeared. They are spherical and stain diffusely. It is difficult to distinguish any nucleoli within them, since they are of about the same size as the mitochondria. There is no apparent change in the mitochondria. The ground substance of the protoplasm presents the same flocculent appearance with a marked tendency toward the production of vacuoles in some of the spores.

Very profound changes now take place. In *Fuligo septica* (Fig. 10) the spores lose every trace of their membranes and degenerate into naked, nucleated masses of protoplasm which are distinctly smaller than in the preceding stage. They also lose their spherical or oval shape and become often quite angular. Their nuclei stain so intensely with iron hematoxylin that it is difficult to make out any structure in them. The ground substance is more homogeneous and stains diffusely and evenly. The appearance of the mitochondria has completely changed. Instead of occurring in the form of large spherules they are now considerably smaller, rod-like and sometimes almost filamentous. They often clump about the nuclei in a manner suggestive of

certain stages in the spermatogenesis of mammals. But they may be quite uniformly distributed through the cytoplasm or else condensed to one side of the nucleus only. Different spores vary greatly in this respect. Sometimes the spores stain so intensely that the mitochondria can only be distinguished with difficulty. The large clear vacuoles and tortuous spaces which occur in some of the spores indicate that it might be worth while to study this material with methods adapted to the demonstration of the vacuolar apparatus. The conditions in *Badhamia* are identical except for the fact that the mitochondria in the spores are much more numerous and filamentous.

Other alterations accompany the formation of the definitive spore capsules which are best seen in sections of *Enteridium rozeanum* (Fig. 11). The capsules are quite complicated in structure. At first sight they seem to be covered with spines but Fig. 11 shows that the spines are in reality the walls of little compartments set upon a homogeneous basement membrane. It often happens that the substance of the spore shrinks away from the membrane. Sometimes this is followed by the partial collapse of the membrane itself. The contents of these mature spores are important. The nuclei seem to be much broken down and traces of them can be distinguished only after careful study. The mitochondria have, almost uniformly, reverted to their original spherical shape and can easily be stained in the usual way with janus green, if the spore contents are squeezed out in a solution of 1:10,000 of the dye.

The entire process of spore formation is subject to great variation in different Myxomycetes. In *Arcyria denudata*, for instance, the process is quite different from that which I have described in *Badhamia*, *Fuligo* and *Enteridium*. The first sign of approaching spore formation is a segregation and encapsulation of comparatively large masses with abundant nuclei and many mitochondria. Instead of being hard to distinguish, the nuclei are quite conspicuous with well-defined chromatin networks and nucleoli. Through successive divisions these multinucleated masses become smaller and smaller. As the final division approaches, the walls become thicker and the nuclei stain less intensely, as is shown in Fig. 12. Division is by mitosis. The

structure of the spores is very intricate and is best brought out when stained with fuchsin and methyl green. The nuclei are large and faintly stained and usually present a solitary spherical nucleolus which likewise stains but faintly. There is often, though not always, present in the cytoplasm an irregular mass of material surrounded by a vacuole. The material can be clearly resolved into acidophilic and basophilic constituents, staining with fuchsin and methyl green respectively. Of the two, the basophilic structures are the most definite and appear in the form of definite spherules suggestive in some measure of chromosomes. But the occurrence of the material in vacuoles is equally suggestive of phagocytosis, which, however, would seem to be unlikely in view of the dense membrane surrounding each spore. Only further study will reveal the nature of the material. Mitochondria are distributed quite evenly throughout the remainder of the cytoplasm and are characterized by their rod-like shape. There is no counterpart here for the changes in the morphology of mitochondria observed in *Badhamia*, *Fuligo* and *Enteridium*.

No special provision is made during spore formation, or in cell division generally, for an equal division of mitochondria, which might be looked for if we regard them as in any sense carriers of heredity. They do not, like the nucleus, change their solubilities or staining reactions during spore formation. This is the more interesting since the resistance of the mitochondria increases progressively in the spermatogenesis of mammals. There is some indication, however, that with spore formation there is a tendency toward a reduction in the amount of mitochondria with relation to the cytoplasm.

Capillitium:

I have not traced the formation of the capillitium from confluent vacuoles as described by Strasburger but my observations of the later stages are of interest in connection with the work of Harper and Dodge ('14, p. 3). In plasmodia of *Hemitrichia clavata* and *rubiformis* fixed in weak Flemming and stained with either safranin, gentian violet and orange G, or with iron hematoxylin, they describe lines running toward the vacuolar tube, where their centers of convergence are marked by a series of granulations (see their Figs. 1–5).

In my own material of the same species, I have been unable to find the radiating lines and perhaps this may be due to the fixative which I have used, but I have observed the granules. The granules in my preparations fixed in Regaud's fluid and stained with iron hematoxylin are almost indistinguishable from the mitochondria which I see in the surrounding plasmodium (Fig. 13). They are of the same shape except in some cases where they are in very intimate contact with the vacuolar membrane and flatten out upon it, which may be due to surface tension. It is important to note that the mitochondria are not illustrated in Harper and Dodge's figures which would lead one to suppose that they have been destroyed by the fixative; so that our conclusion is warranted that the granules which surround the vacuole and constitute the termini of the lines are more resistant than the mitochondria to fixation. It is probable that the destruction of the mitochondria may have been occasioned by the acetic acid in the Flemming's fluid. We know that plasts generally are more resistant to acetic acid than are true mitochondria, which would lead us to suppose that the granules in question are plast-like. This interpretation falls well in line with Harper and Dodge's explanation of the significance of the radiating lines. They regard them as "pathways by which materials are brought in from the surrounding cytoplasm." I cannot agree with them, however, in their interpretation of the significance of the nuclei as morphogenic factors. I would be inclined, on the contrary, to regard the granules as plast-like and perhaps truly formative, as in the higher plants.

Furthermore, Harper and Dodge ('14, p. 7) have described certain interesting formations within the lumen of the capillitial tube. They say that:

"The granular material in the interior of the capillitial thread (Figs. 1 and 2) becomes less as the wall thickens and the spirals appear, and as the thread matures it practically disappears (Figs. 3 and 4). There is, of course, no evidence that granular material as such passes from the interior of the thread into the forming spirals. We are inclined to suspect that the stainable granules in the interior of the thread are precipitation products formed in fixation, and that in the living condition the capillitial

cavity contains only materials in solution in the cell-sap. These materials may be used up in the formation of the capillitial wall and spirals so that in late stages no such precipitation products are formed."

In the first place my observations show, contrary to Harper and Dodge, that the granular material is quite abundant in well formed capillitial threads. In Fig. 17 of *Arcyria denudata* the granules are very numerous and occupy almost the whole interior of the thread. In fact I have never found them to be so abundant in early stages in the formation of the capillitial threads in *Hemitrichia clavata*. Neither have I found any indication that granular material as such passes from the interior of the thread into the forming spirals (see Figs. 13, 15, 17, 18 and 19). It is hard for me to believe that these granules are precipitation products formed by fixation. It will be noted that they are often of astonishingly uniform size and shape which one would not expect in products of precipitation or coagulation. Moreover they are often absent or isolated or distributed evenly over the interior of the thread showing little tendency to clump, which one would likewise expect in the case of products of fixation. They do resemble mitochondria very closely. Their rod-like and even filamentous shape is well illustrated in my figures. They are often arranged in rows, suggestive also of mitochondria. They are usually about the same size as mitochondria but they are sometimes larger, as shown especially in Fig. 17. Their staining reactions also are suggestive of mitochondria, for they take the fuchsin as well as the iron hematoxylin, after fixation in Regaud's fluid. Nevertheless we cannot consider them to be mitochondria because they occur within the capillitial tubes, quite apart from nuclei and from protoplasm. It would appear more probable that they constitute merely a part of the material brought in, which is useless in the formation of the capillitial wall.

Harper and Dodge refer to two types of nuclei at this stage in *Hemitrichia clavata* and I have been able to confirm their finding as illustrated in Fig. 19. It will be seen that the majority of the nuclei are large and pale and have definite nucleoli. Some, however, are smaller and much more intensely stained with the hematoxylin: these Harper and Dodge regard as undergoing

degeneration. They are distributed quite evenly throughout the plasmodium which definitely precludes the possibility that they may result from mechanical injury. In preparations stained with fuchsin and methyl green, the small nuclei take the fuchsin to a remarkable degree, behaving just like the nuclei of the spores, which stain in precisely the same way. This points, perhaps, to the conclusion that these small nuclei are undergoing differentiation with a view to spore formation.

Sporangium Wall and Hypothallus:

My observations do not bear upon the question of the mode of formation of the sporangium wall and hypothallus. In all probability both of them are differentiations or secretions of the plasmodium as is generally supposed. I have found no indications that the mitochondria play any part in their formation. They are at first gelatinous and afterwards become membranous. A good account of sporangium formation in *Trichia* and *Arcyria* is given by Kranzlin ('07, p. 179).

With this progressive differentiation of spores, capillitium, sporangium wall and hypothallus, there is a distinct and gradual alteration in the plasmodium itself. At first it usually contains comparatively large quantities of debris, the more solid portion of which is left behind in its path and persists to some extent in the hypothallus. There is a further segregation with the formation of differentiated products, the end result being that the protoplasm of the spores is comparatively free of foreign material.

Discussion.

We are inclined to divide living organisms into two groups, plants and animals, and perhaps unconsciously to assume that this classification is sufficient. It is interesting to find that the Myxomycetes, or slime moulds, cannot be dismissed so easily, for they partake of the properties which we have been inclined to regard as distinctive of plants, on the one hand, and of animals, on the other. Great difference of opinion is manifest in the literature. At first they were considered to be plants and were called *Myxogastres*, in 1829, by Fries, who grouped them among the fungi. The word Myxomycetes also indicates their fungous-

like properties. With the discovery of the plasmodium, opinion changed and the term *Mycetozoa* was introduced. Even now there is absolutely no concensus of opinion on the subject of their relationships. Coulter, Barnes and Cowles ('10, p. 1) place them tentatively among the first of the *Thallophytes*. Chamberlain ('15, p. 152) groups them with the *Schizophytes*. Torrend[1] and Schintz[2] conclude that the *Myxomycetes* are related to *Fungi* rather than to animals. And finally Osborn ('11, p. 339) Schwartz ('14, p. 238), Harper ('oc, p. 235) and others associate them with the Plasmodiophoraceæ, Chytrideæ and Acrasieæ; while Maire and Tison[3] relate them to the Sporozoa, Elliott[4] to animals generally, by reason of their feeding reactions, and Parker and Haswell ('97, p. 61) classify them as intermediate between the Rhizopoda and the Mastigophora.

We may summarize their plant-like features as follows: In the first place they form brilliantly colored fungous-like masses strongly suggestive of true fungi. The capillitial threads which are found in the sporangia frequently play an important part in the dispersal of the spores and remind one of the elaters of liverworts. If Harper and Dodge ('14, p. 9) are correct, however, there would appear to be a closer analogy between the capillitium and the protozoan endoskeleton, since they believe them both to be formed by a process of intraprotoplasmic secretion. They bear also a certain superficial resemblance to the puff-balls, which have likewise a mass of spores supported by a capillitium-like framework and contained in a sporangium. Pinoy ('08, p. 630) records a sexual dimorphism in *Didymium* which reminds him of the condition in Mucor as described by Blakeslee. Moreover the supposed presence of cellulose in the sporangium walls, the spore walls and the cyst walls of the sclerotium indicate a plant affinity. And finally the possession of well-defined spores is cited as evidence that they should be regarded as plants.

Yet they apparently resemble animals just as closely and for this reason they have been called "Mycetozoa" (fungus-animals),

[1] Reviewed in *Jour. Roy. Micr. Soc.*, 1910, p. 221.
[2] *Jour. Roy. Micr. Soc.*, 1914, p. 292.
[3] *Jour. Roy. Micr. Soc.*, 1909, p. 626.
[4] *Jour. Roy. Micr. Soc.*, 1917, p. 500.

a term which indicates a compromise, and is perhaps preferable. We may enumerate briefly their many points of resemblance to members of the animal kingdom. It has already been mentioned that the plasmodium behaves to all intents and purposes like a gigantic amœba. It moves freely from place to place, burrows deeply in rotten wood and into the substrata when it is necessary to obtain nutriment, and, most significant of all, it is capable of phagocytosis. That is to say, it can actively devour and digest bacteria and other foreign bodies which it is able to engulf. In the total absence of chlorophyll, the green pigment so characteristic of plants generally, and the accompanying saprophytic behavior, they resemble the fungi, bacteria and other saprophytic plants, on the one hand; and the whole animal kingdom on the other, with but few exceptions. Following a study of the feeding habits of *Badhamia* Elliott[5] looks on Mycetozoa as parasites, more animal than vegetable. The swarm cells with their flagella and food vacuoles, their motility and power to multiply by fission call to mind the Flagellata.

To these points of similarity to animals may now be added the mitochondria, which I have found to occur in all the species of Myxomycetes which I have examined, numbering ten or more. The mitochondria observed are identical, so far as can be ascertained, with the mitochondria in the higher plants and in the whole animal series from the protozoa to man. The point is, that in some lower plants, the mitochondria are apparently totally absent or else quite different from those which I have described in the Myxomycetes. They have not been described in the Cyanophyceæ; in the bacteria their presence is doubtful (E. V. Cowdry '16a, p. 433), and in the Chlorophyceæ they have been found in but few forms: Guilliermond ('13, p. 86) thinks that here the enlarged chloroplast takes over their function. So that the mitochondria of the Myxomycetes approximate far more closely to the mitochondria of animals than to those of the lowest plants.

The discovery of mitochondria in the Myxomycetes extends our knowledge of the extraordinary breadth of distribution of these granulations in living matter. I have already shown ('17, p. 225) that, so far as our present methods of technique go,

they are identical in animals and in plants. Champy's ('11, p. 154) statement that "I would not regard as living a cytoplasm which does not contain mitochondria" is rather radical in view of our knowledge of the structure of the lowest plants. The bacteria and the Cyanophyceæ are of special interest in this connection. Furthermore, it is common to find comparatively large stretches of protoplasm in certain of the Myxomycetes which contain no mitochondria. While we cannot say how active this protoplasm is we cannot regard it as totally inert and lifeless.

Concerning the continuity of mitochondria in the Myxomycetes it may be said that they invariably occur in the plasmodia as well as in all stages of spore formation, even to the adult spore. While I have not yet studied the swarm spores, it is extremely probable that mitochondria occur in all stages of the life cycle. No indications were observed of *de novo* formation of mitochondria though it is highly improbable that if such occurred they would have been detected. It seems unnecessary to assume, as some workers have done, that we must find mitochondria grading into the invisible in order to demonstrate a *de novo* origin, because it is possible that the mitochondrial aggregate must attain to a certain size before acquiring characteristic density and staining reactions.

The cyclical changes in the morphology of the mitochondria suggest similar changes which have already been described, long since, in higher forms. The most striking of these is the change from the large spherical mitochondria of the youngest spores to the smaller rather rod-like ones of those which are more mature. They resume their granular condition in the mature spores. They are often rod-like and arranged parallel to the direction of the current in streaming protoplasm of active plasmodia. Their clumping about the nucleus and their whole behavior make it very plain that their morphology and distribution are governed by the same laws here which operate in animals and in the higher plants, whatsoever they may be.

It is interesting to note, *a propos* of current statements to the effect that the mitochondria are transformed into cellular differentiations, that, so far as can be ascertained, they play no

active part in the formation of the sporangium wall, the complicated spore capsules and capillitia, the hypothallus, the pigment and lime deposits of the Myxomycetes.

The occurrence of typical mitochondria in the active plasmodia, crawling from place to place, as well as in all stages in spore formation even to the fully mature spore, surrounded as it is with a thick restraining horny capsule must indicate one of two things: either that the mitochondria are active in some of these locations and passive in others or else that they take part in the activities of the cell in all stages. The second supposition is usually granted by workers on mitochondria in higher forms. It is apparent, then, that this study of the Myxomycetes materially supports the general supposition that the mitochondria are concerned in some fundamental vital process common to all living matter, perhaps with protoplasmic respiration and possibly with growth.

Conclusions.

1. Mitochondria occur in *Arcyria*, *Badhamia*, *Ceratiomyxa*, *Cribraria*, *Enteridium*, *Fuligo*, *Hemitrichia*, *Lycogala*, *Stemonitis* and probably in all other Myxomycetes.

2. The mitochondria in Myxomycetes so far as can be ascertained differ in no wise from those occurring in the majority of plants and in all animals.

3. The Myxomycetes resemble the lower animals much more closely than they do the lower plants with respect to their mitochondrial content.

4. The Myxomycetes offer a unique opportunity in many ways for the experimental study of mitochondria.

Bibliography.

Benda, C.

'01 Die Mitochondriafärbung und andere Methoden zur Untersuchung der Zellsubstanzen. Verh. d. Anat. Gesell., pp. 155–174.

Chamberlain, C. J.

'15 Methods in plant histology. Univ. of Chicago Press, 314 pp.

Champy, C.

'11 Recherches sur l'absorption intestinale et le rôle des mitochondries dans l'absorption et la sécrétion. Arch. d'Anat. micr., T. 13, pp. 55–170.

Coulter, J. W., C. R. Barnes and H. C. Cowles.

'10 A Textbook of Botany. Vol. 1, Morphology and Physiology. American Book Co., New York, 484 pp.

Cowdry, E. V.

'16a The general functional significance of mitochondria. Amer. Jour. Anat., Vol. 19, pp. 423–446.

'16b The structure of chromophile cells of the nervous system. Contributions to Embryology, No. 11, pp 27–43.

Cowdry, N. H.

'17 A comparison of mitochondria in plant and animal cells. BIOL. BULL., Vol. 33, pp. 196–228.

Guilliermond, A.

'13 Sur la signification du chromatophore des algues. C. rend. Soc. Biol., T. 75 pp. 85–87.

Harper, R. A.

'00 Cell and nuclear division in Fuligo varians. Bot. Gaz., Vol. 30, pp. 217–251.

Harper, R. A., and B. O. Dodge.

'14 The formation of the capillitium in certain Myxomycetes. Ann. Bot., Vol. 28, pp. 1–18.

Kranzlin, Helene.

'07 Zur Entwicklungsgeschichte der Sporangien bei den Trichien und Arcyria. Arch. f. Protisten., Bd. 9, pp. 170–194.

Osborn, T. G.

'11 Spongospora subterranea. Ann. Bot., Vol. 25, pp. 327–341.

Parker, T. J., and W. A. Haswell.

'97 Textbook of Zoology, Vol. 1, London, Macmillan and Co., 779 pp.

Pinoy, E.

'08 Sur l'existence d'un dimorphisme sexuel chez un *myxomycete*, didymium nigripes, Fries. C. rend. Soc. Biol., T. 74, pp. 630–631.

Regaud, Cl.

'10 Étude sur la structure des tubes seminiféres, etc. Arch. d'Anat. micr., T. 11, pp. 291–433.

Sapehin, A. A.

'15 Untersuchungen über die Individualität der Plastide. Arch. Zellforsch. Bd. 13, pp. 319–398.

Schwartz, E. J.

'14 The Plasmodiophoraceæ and their relationship to the Mycetozoa and the Chytridiæ. Ann. Bot., Vol. 28, pp. 227–240.

Explanation of Plate I.

All the figures have been drawn with Zeiss apochromatic 1.5 mm., compensating ocular 8 and camera lucida. They have been reproduced without reduction so that the magnification as they now appear on the plates is 2,600 diameters.

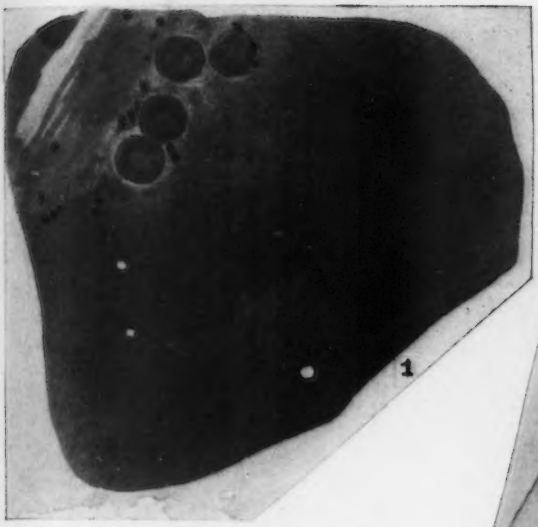
Fig. 1. *Enteridium rozeanum* fixed in Regaud's fluid and stained by the Benda method. The mitochondria are stained a dark purplish-blue color against a pink background. Plasmodium showing a large area of apparently homogeneous protoplasm, a few spherical nuclei and some mitochondria, are seen to one side. The ground substance shows a tendency to be more vacuolated and to stain fainter in the vicinity of the nuclei.

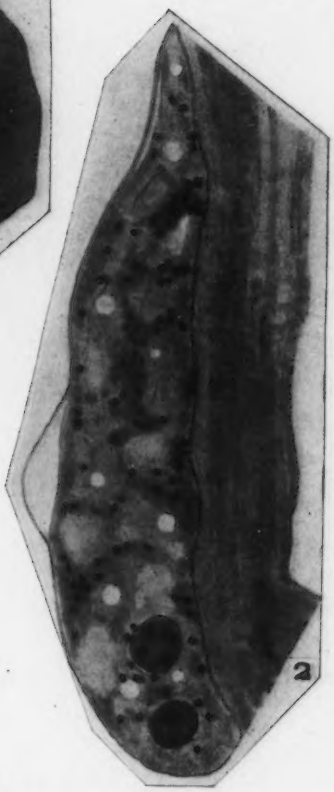
Fig. 2. *Lycogala epidendrum* fixed in Regaud's fluid and stained with iron hematoxylin. The mitochondria are stained bluish-black against a gray back ground. A portion of the plasmodium crawling upward toward the æthalium on the meshwork of the hypothallus.

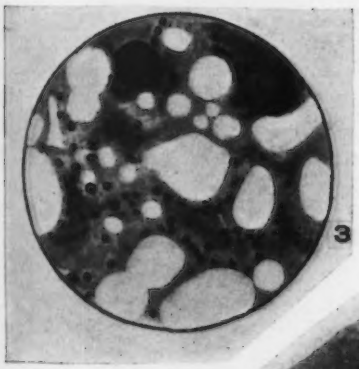
Fig. 3. *Enteridium rozeanum* same fixation and stain as Fig. 1. Likewise a portion of the plasmodium greatly vacuolated. The nuclei are stained intensely and the mitochondria are often grouped about them as well as about the vacuoles.

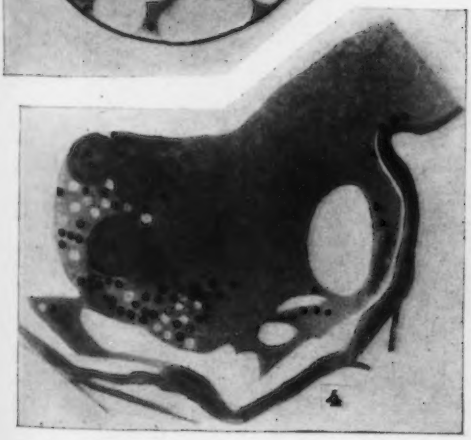
Fig. 4. The same showing quite well differentiated fibrous structures which often appear to be of the nature of septæ separating the plasmodium into different areas.

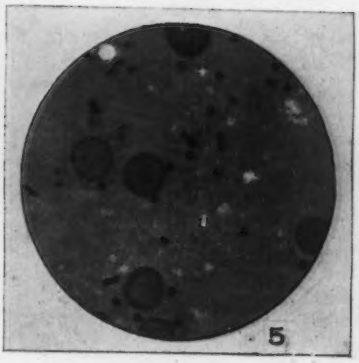
Fig. 5. Another part of the same plasmodium with scattered nuclei and mitochondria of various forms. The ground substance shows an indistinct vacuolation.











N. H. COWDRY.

Explanation of Plate II.

Fig. 6. *Enteridium rozeanum* fixed in Regaud's fluid and stained by the Benda method, mitochondria blue-black and ground substance pink. Another septum is shown at the side. The mitochondria occur in distinct clumps sometimes about the nuclei. The apparent differences in the staining reactions of the mitochondria simply indicate depth in the preparation.

Fig. 7. *Fuligo septica* fixed in Regaud's fluid and stained with iron hematoxylin, mitochondria blue-black and ground substance gray. Immature spore with a faint nucleus and conspicuous mitochondria.

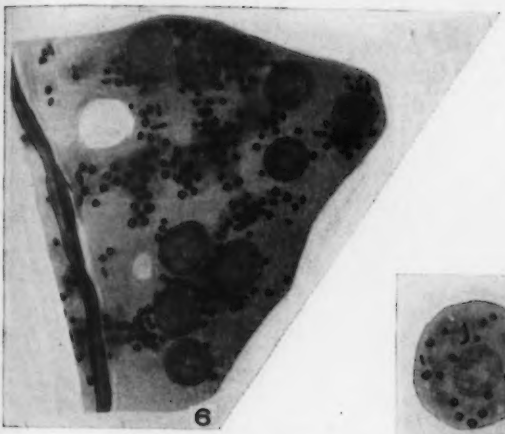
Fig. 8. *Badhamia* ————, fixed and stained in the same way. It shows the first stages in spore formation. The nuclei are indistinct; the mitochondria show progressive clumping and the spore membranes are gradually formed.

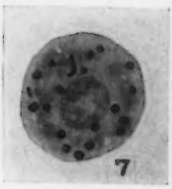
Fig. 9. *Fuligo septica*, same fixation and stain. More mature spores of smaller size, without a distinct cell membrane, with strongly stained nuclei and more rod-like mitochondria. The mitochondria often closely approximate to the nucleus. The ground substance is more homogeneous than in the preceding stage and more deeply stained.

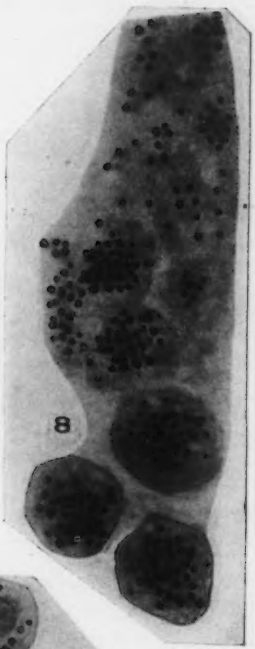
Fig. 10. *Fuligo septica* fixed and stained in the same way showing rather later stages in spore formation than Figs. 7 and 8. The spores are surrounded with a definite membrane. They have faintly staining nuclei and spherical mitochondria. One of them is rather more advanced.

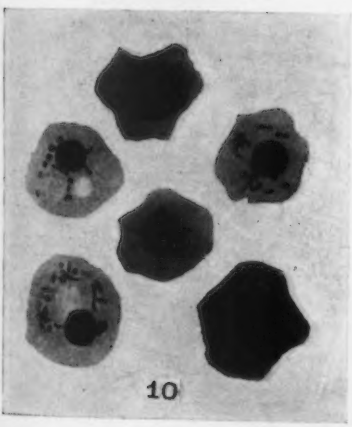
Fig. 11. *Enteridium rozeanum* same fixation and stain. Quite advanced spores with definite capsules. The nuclei are faintly stained and the mitochondria spherical and in one case clumped near the nucleus.

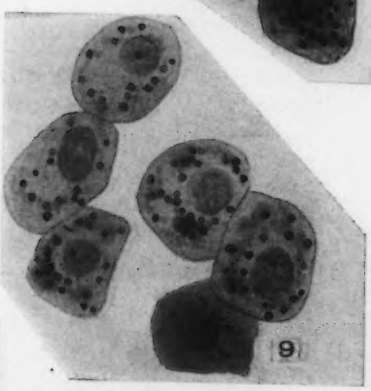
Fig. 12. *Arcyria denudata* fixed in Regaud's fluid and stained with fuchsin and methyl green. The mitochondria are crimson against a greenish background. One cell not finally divided containing three nuclei and two others each containing a mass of material in a vacuole. The material consists of a basophilic part, staining green, definitely delimited in the form of spherules, and of a more irregular acidophilic mass staining red.

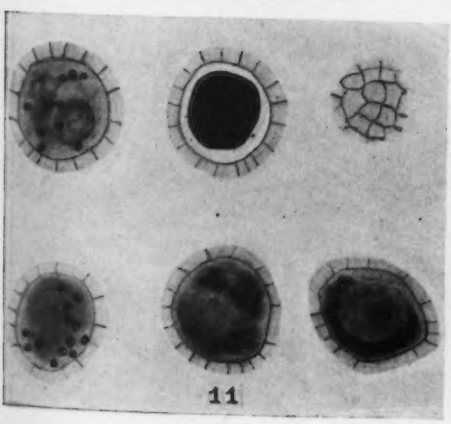


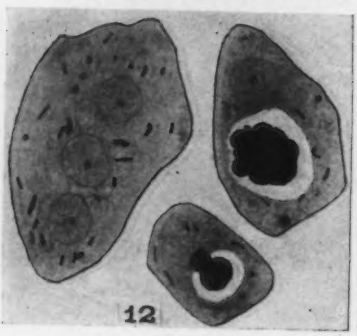












R. H. COWDRY.

Explanation of Plate III.

Fig. 13. *Hemitrichia clavata* fixed in Regaud's fluid and stained with iron hematoxylin. The mitochondria are blue-black and can with difficulty be distinguished from the granulated ground substance. Plasmodium, within the sporangium, showing an early stage in the formation of the capillitium. The spirals are beginning to appear.

Fig. 14. *Arcyria denudata* fixed and stained in the same way. These cells are in the stalk, next the envelope. They will apparently take no part in spore formation. They show indications of cytolysis and contain but few mitochondria.

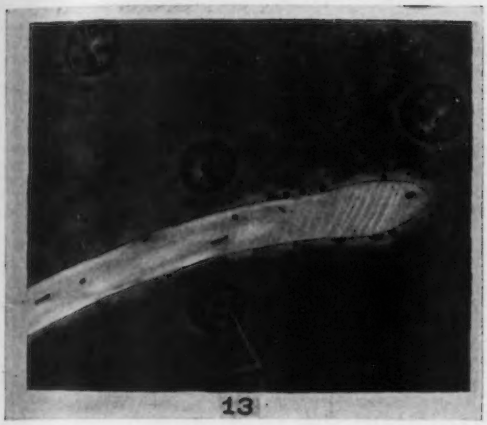
Fig. 15. *Hemitrichia vesparium* fixed and stained in the same way. Spores and cross and longitudinal sections of capillitium.

Fig. 16. *Hemitrichia vesparium*, same fixation and stain. A portion of the plasmodium flowing between the meshwork of the hypothallus. The mitochondria are elongated in the direction of movement. There is also a faint striation in the ground substance in the same direction. Some of the nuclei have well marked nucleoli.

Fig. 17. *Arcyria denudata* fixed in Regaud's fluid and stained with fuchsin and methyl green. The mitochondria are crimson. There are two immature spores which apparently contain a comparatively large amount of golden yellow pigment. The terminal enlargement of the capillitial thread also contains a series of granulations staining just as the mitochondria do.

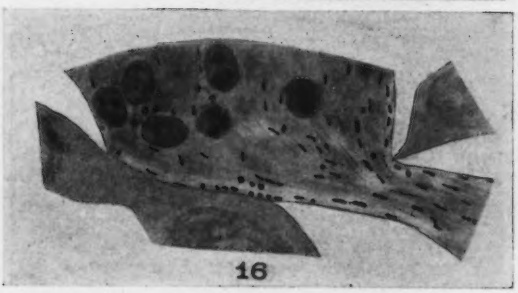
Fig. 18. *Hemitrichia clavata* fixed in Regaud's fluid and stained with iron hematoxylin showing also internal granulation.

Fig. 19. *Hemitrichia clavata* fixed and stained in the same way. Plasmodium with cross section of capillitial thread and two types of nuclei, the smaller ones staining darkly and the larger ones lightly.


13

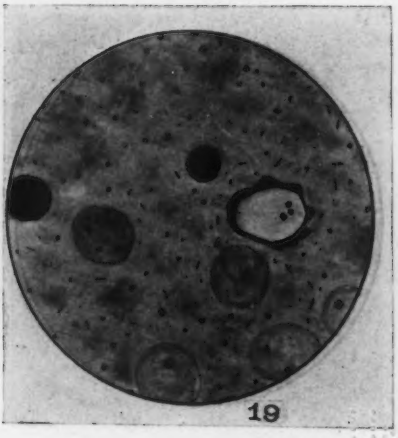
14

15


16

17

18


19

A CONTRIBUTION TO THE PHYSIOLOGY OF WING DEVELOPMENT IN APHIDS.[1]

GEORGE O. SHINJI,

UNIVERSITY OF MISSOURI, COLUMBIA, MO.

CONTENTS.

		Page.
I.	Introduction	95
II.	Experiments with the Rose Aphid	97
III.	Experiments with other Species of Aphids	102
IV.	Period during which the Wing Development may be Controlled	104
V.	Strength of Magnesium Salts needed to Produce the Winged Aphids	105
VI.	Physical Factors:	
	1. Temperature	106
	2. Wilting of the Plant	107
VII.	Morphological Studies	108
VIII.	General Discussion	110
IX.	List of Aphids Experimented with	113
X.	Summary	114
XI.	Literature Cited	116

I. INTRODUCTION.

There are at least two closely related and equally interesting phenomena observed in the aphids, or plant-lice as they are commonly called. These are the development of apterous or alate forms, or both, as the offspring of either a winged or apterous viviparous female, and the parthenogenesis exhibited by them. The phenomena are not the result of alternation of generations, for it has been found by Slingerland (1893) and others that under certain favorable conditions a species of aphid might continue multiplying for a period of several years without producing a single male or winged individual.

Several attempts have been made to explain the phenomenon of wing-development in aphids. Shortage of food supply, it is believed, will usually produce winged aphids so that they may fly away to other plants where the supply of food has not been

[1] A thesis submitted in partial satisfaction of the requirements for the degree of Master of Science in the Graduate School of the University of California, April, 1913.

exhausted. Climatic changes, especially the approach of cooler weather, humidity, the wilting of host plants and many other factors have been regarded as possible causes of the appearance of the winged aphid. All explanations, however, have been advanced as the results of observations rather than as the results of experimental work.

The first experimental study of the problem was undertaken by Professor W. T. Clarke, of the University of California, in 1901. He planted a rose twig bearing an apterous parthenogenetic female in each of a series of four-ounce tumblers. These he filled with washed sand, moistened with solutions of several salts or pure water. By this method, he could charge with any desired solution the sap-sucking insect through the plant. He was influenced, so he states, in his choice of chemicals by the chemical analysis of the ingredients of plants. Professor Clarke used sodium hydroxide, sodium phosphate, magnesium sulphate, magnesium chloride, and distilled water as a check. As a result of such experiments, he found that among these substances only the magnesium salts would produce winged aphids. Changes of temperature, he suggested, may not be a factor in the development of the wing in aphids.

In 1908 Professor C. W. Woodworth expressed his views on the problem. He stated that he believed that the wilting of plants might help develop the wing by retarding the growth of other parts and giving an even chance for the wing buds to develop.

Mr. J. D. Neiils, who studied the problem in 1912, not only confirmed the findings of Clarke, but also pointed out a new fact that three days were usually required for the magnesium salts to produce a large number of winged aphids. Further, he found that the young aphids become winged, if they are subjected to the magnesium solutions within three days after birth.

Professor Clarke, and also Mr. Neiils, worked on a single species, namely, the common rose aphid, using the limited series of chemicals above mentioned. In order to determine whether other salts than those of magnesium would have a similar effect on the production of winged aphids, and also to determine whether the results of Clarke and Neiils were applicable to other species of aphids, I undertook an experimental study of the problem.

Before going further, the writer wishes to acknowledge his indebtedness to Professor Woodworth, at whose suggestion the work was begun. His hearty thanks are also due to Professor E. Van Dyke, who, jointly with Professor Woodworth, gave valuable criticism and helpful suggestions during the progress of the investigation.

II. Experiments with the Rose Aphid.

These were partly to verify the findings of Professor Clarke and partly to make a more extensive test of the action of chemicals. The method of experimentation was that adopted and carried out by Professor Clarke. Sharp, clean sand was washed several times in running water, and then left to soak over night. The following day it was washed again with distilled water and after drying was ready for use. Four-ounce tumblers were then filled to one half of their depth with this sand and moistened with solutions of various chemicals. In each of these tumblers, a rose twig bearing from one to three viviparous females, usually apterous, was planted. Though Mr. Neiils pointed out the fact that three days are usually required for the magnesium salts to produce the largest number of winged aphids, it is not always necessary that the mother aphids be transferred to the twig that number of days after it is planted, to see the effect of the salts, for, as is also stated by Mr. Neiils, the young aphids become winged if they are subjected to magnesium salts within three days after birth. Consequently, I used the method previously described. A careful watch was kept of the twigs and when winged forms appeared, they were taken away, and the records kept of their appearance, as well as of the total number of young born. In my later experiments, the record was taken only once, when the first born had acquired the wings, because I had found that the tendency of the young aphids to produce wings could be accurately determined a certain number of days after birth.

I had thought that some other substances might also produce winged aphids. This belief was suddenly strengthened when I found, late in November, that a rose twig that was planted in an old tin can containing ordinary water, was infested with 126

winged and 2 apterous forms. This, I thought, was due to the presence of either tin or iron, because ordinary water, as will be seen elsewhere in the table, never produces more than 5 per cent. of winged forms. Consequently, I tested several salt solutions of the heavier metals and obtained the results which not only confirmed my belief, but also incidentally led to the finding of several new salts which could develop winged aphids.

Sugar and urea were also used in order to determine the effect of electrolytes as well as osmotic pressure. Urea never, or very rarely, produced winged individuals. The action of cane sugar caused rather confusing results, because the young ones produced on twigs, placed in the sugar solution that had been standing for a few days, remained apterous, while those on twigs in fresh sugar solution produced winged forms. These apparantly contradictory results, it was first thought, might be due to the decomposition of sugar by fermentation. That this explanation may be correct is indicated by the fact that both alcohol and acetic acid always produced apterous forms. From December 8th on, the practice was followed of transferring the twig to a fresh solution every three days with uniform results.

When a stronger solution, about $m/20$ of strontium bromide or strontium chloride was used, the tips of the twigs would dry out in the course of from 36 to 48 hours. Many aphids would fall off the twigs; those staying on the twigs would change to a dark salmon color, lose water, and finally dry upon the twig. Even in a solution of $m/100$–$m/300$ of strontium bromide, the twigs planted in it became darker in color and emitted the characteristic odor of strontium salts. About 99 per cent. of the young aphids remaining on the twig developed no wings.

Calcium chloride produced the same, but a weaker effect on the twig and also on the aphids. All aphids stayed on the twig charged with $m/50$ solution of the salt, but remained apterous.

Owing to the very poisonous nature of the chemicals used, often the entire number of the young born on a twig charged with the solutions of mercury, lead or copper salts, would drop off before it could be determined whether they were winged or not. But the fact that some of them, happening to remain long enough, produced wings indicated that the solution had the wing-develop-

TABLE I.

EXPERIMENTS WITH ROSE APHIDS.

Date.	Chemicals.	Total No. of Winged Individuals.	Total No. of Apterous Individuals.
Up to October 20...	$CaCl_2$	2	83
	H_2O, distilled	0	62
	H_2O, tap	7	112
	KCl	0	58
	Na_2CO_3	0	38
	NaOH	1	153
	$MgSO_4$	182	4
	Sugar	18	34
	$SrCl_2$	1	40
	Urea	2	32
October 22.....	$CaCl_2$	0	42
	H_2O, distilled	0	24
	KCl	0	42
	$NaHpO_4$	0	28
	$MgSO_4$	84	1
	$SrCl_2$	0	14
	Sugar	36	25
	Urea	0	24
	$MgSO_4$—NaOH	22	2
	$MgSO_4$—Na_2CO_3	30	5
	$MgSO_4$—Na_2CO_3	2	16
	Acetic acid	0	52
November 11.....	Ordinary water	2	24
	KCl	0	27
	NaOH	0	52
	$MgSO_4$	142	1
	Mg citrate	28	1
	Sugar	82	0
	$SrCl_2$	1	14
	Tin can	38	3
	NaOH—$MgSO_4$	48	11
December 2.....	Alcohol	1	48
	$CaCl_2$	0	14
	H_2O	0	48
	KCl	0	20
	NaOH	0	48
	Na_2CO_3	1	143
	$MgSO_4$	12	0
	Mg citrate	24	0
	Tin can H_2O	62	0
	Tannin	0	14
	$SrCl_2$	0	28
	Creek water	3	87
	Urea	0	18
December 18.....	$SrCl_2$	33	1
	$CuSO_4$	12	0
	$Hg(NO_3)_2$	6	1
	$NiSO_4$	86	0
	$ZnCl_2$	38	1
	$SnCl_4$	92	0
	$AgNO_3$	11	0
January 20, 1913...	$Hg(NO_3)_2$		
	$MgSO_4$	8	0
	$NiSO_4$	141	0

Date.	Chemicals.	Total No. of Winged Individuals.	Total No. of Apterous Individuals.
January 20, 1913...	$PbCl_2$	2	0
	$SbCl_3$	18	2
	$SnCl_4$	148	4
	Na_2CO_3	0	53
	NaOH	0	38
	Urea	0	14
	Sugar	1	85
	Alcohol	0	62
	Tannin	1	14
	Alum	1	82
	H_2O	0	28
February 7	$CuSO_4$	5	0
	KCl	0	59
	H_2O	0	48
	$NiSO_4$	62	0
	NaOH	0	48
	$SnCl_4$	111	1
	$ZnCl_2$	2	
February 21	$CaCl_2$	0	52
	$CuSO_4$	5	1
	$AgNO_3$	12	0
	$HgCl_2$	17	4
	H_2O	1	148
	KCl	1	14
	$SrCl_2$	0	29
	Na_2CO_3	0	24
	$SnCl_4$	184	1
	$MgSO_4$	17	1
	$PbCl_2$	10	2
	$ZnCl_2$	8	1
	Alcohol	1	42
	Acetic acid	0	14
March 5	$AgNO_3$	2	0
	$CaCl_2$	0	33
	KCl	2	54
	H_2O, surface	2	142
	H_2O, distilled	0	22
	$HgCl_2$	8	1
	$MgSO_4$	31	1
	$NiSO_4$	110	2
	Na_2CO_3	0	87
	$SrCl_2$	0	42
	Sugar	1	15
	Urea	2	18
March 20	$AgNO_3$	14	0
	$CaCl_2$	0	48
	H_2O, distilled	6	114
	KCl	0	61
	$SrCl_2$	0	32
	$MgSO_4$	36	1
	Mg citrate	44	0
	Na_2CO_3	1	20
	$NiCl_2$	98	1
	$SnCl_4$	112	3
	Urea	1	16
	Sugar	218	1

Date.	Chemicals.	Total No. of Winged Individuals.	Total No. of Apterous Individuals.
April 3	$AgNO_3$	12	0
	Alcohol	0	136
	$CaCl_2$	6	62
	$CuSO_4$	12	0
	KCl	0	47
	H_2O, tap	2	48
	$MgSO_4$	32	0
	$NiSO_4$	182	1
	NaOH	0	84
	$NaCO_3$	0	92
	$SrCl_2$	0	24
	$SnCl_4$	116	0
	Sugar	118	1
	Urea	0	31
	Alum	0	16
	Peptone	1	15
	Sugar + $MgSO_4$	0	24
	Sugar + Na_2CO_3	1	82
	$MgSO_4$ + NaOH	0	62
	Sugar + NaOH	0	40
	Sugar + NaOH	0	12
	Sugar + NaOH	2	12
	Sugar + NaOH	0	42

The following is the summary of the foregoing data.

	Winged Individuals.	Apterous Individuals.
$AgNO_3$	51	0
$CuSO_4$	34	1
$HgCl_2$	31	6
$NiSO_4$	955 ?	5
$SbCl_3$	41	5
$PbCl_2$	12	2
$SnCl_4$	579	8
$ZnCl_2$	49	2
Mg salts	840 ?	9
Sugar	512	4
Alcohol	288	2
Alum	3	34
Acetic acid	0	67
Na salts	2	1029
Ca salts	1	433
K salts	3	324
Sr salts	1	220
Tannin	1	14
Urea	5	153
Water, distilled	0	394
Water, tap and creek	17	461
Peptone		15

ing qualities. Only a few drops of the normal solutions of these salts were added to the 5 c. c. of distilled water, in most of my experiments.

The above results not only verified the findings of Professor Clarke, but also led to the discovery of new substances. In addition to the magnesium salts, salts of antimony, nickle, tin, mercury, lead, zinc and also sugar were found to produce winged aphids; while alcohol, acetic acid, alum, tannin, salts of strontium, potassium and calcium, and also urea were shown to have no effect. These substances may, therefore, be classified into wing-developing and non wing-developing substances, respectively, according to their action on the wing buds. To the former class, belong the salts of alkalis and the alkaline earths, while to the latter belong all of the other salts mentioned, as well as sugar.

III. Experiments with Other Species of Aphids.

The studies thus far mentioned concerned only the common rose aphis. Do these substances have a similar effect upon other species of aphids? I, therefore, instituted another series of experiments, using the same methods as were employed in the course of the development in the rose aphid.

The following results were obtained (Table II.):

As is shown in this table, the results obtained with these aphids are not different from those obtained with the rose aphids, *i. e.*, alkali salts and salts of alkaline earth, together with distilled water, tannin, alcohol and acetic acid produced no winged aphids, while the rest of the salts and also sugar produced winged forms in these species, as they did in the rose aphids.

The periwinkle aphids, as will be noticed in the table, never yielded one hundred per cent. of winged individuals with ordinary strengths of wing-producing substances; *e. g.*, a fifteen per cent. solution of sugar or $m/40$ of magnesium sulphate. Subjection to a very weak solution of the alkali salts, or even to distilled water, did, on the other hand, completely suppress the development of the wings. Thus, although the number of the winged forms produced on twigs charged with wing-producing substances was small, their appearance must be accredited to the presence of these substances. The common aphids on Sonchus, on the mustard and on the German ivy (*Senecio* sp.) were found to develop their wings after subjection to very weak solutions of

TABLE II.

Date.	Substances.	Species of Aphids.							
		Mustard Aphid.		Sonchus Aphid.		Periwinkle Aphid.		Senecio Aphid.	
		No. of Winged Forms.	No. of Apterous Forms.	No. of Winged Forms.	No. of Apterous Forms.	No. of Winged Forms.	No. of Apterous Forms.	No. of Winged Forms.	No. of Apterous Forms.
Up to Oct. 20	H_2O	228	2						
	KOH	2	48						
	$MgSO_4$	145	2						
	Sugar	24	2						
	H_2O	2	62						
	$MgSO_4$	12	0						
	Sugar	51	0						
Nov. 11	H_2O, tap			1	27				
	KCl			0	16				
	$MgSO_4$			37	0				
	Sugar			72	0				
Nov. 25	$MgSO_4$	82	1	40	0	8	10		
	Sugar	28	0	27	27	7	12		
	H_2O			0	12	0	42		
Dec. 4	H_2O	2	24	1	28	0	28		
	$MgSO_4$	25	1			8	6		
	Sugar	78	0	42	0	12	10		
	Tin can and tap water					10	30		
Feb. 27	H_2O	4		4	41	0	12	4	41
	$NiSP_4$			15	0	2	8		
	$MgSO_4$			12	0	8	11		
	$SnCl_4$			20	2	14	6		
	KCl			0	14	0	10		
	$CaCl_2$			0	32	1	18		
March 14	$CaCl_2$							1	12
	$MgSO_4$							20	0
	$SnCl_4$							112	2
	H_2O							0	81
March 27	H_2O			0	28	0	72	2	142
	KCl					0	72	0	129
	Na_2CO_3					6	10	0	60
	$CaCl_2$							1	28
	Sugar							12	0
	Urea							0	6
	$MgSO_4$					12	45	28	1

any of the wing-producing substances. Thus, it is evident that there exists a variation in the degree of susceptibility among the species of aphids.

It might also be well to add here that in several species of aphids belonging to the tribe Chaitophorini no apterous viviparous females have as yet been found. Such, for example, is the

case with the chestnut aphis (*Myzocalis castanæ*), the ulmus aphis (*M. ulmi*), the oak aphis (*M. quercus*, etc.), the birch aphis (*Euceraphis betulæ*), the linden aphis (*Eucallipterous tiliæ*) and others. These species do not grow on any other host plant except their specific ones, nor are they easily reared on cuttings, or even on young potted seedlings. Although the writer failed to produce apterous forms of these Chaitophorini, the development of wings in these species is no doubt due to the nature of the host and also due to the feeding habits of the aphids, for all the young are apterous.

The aphids infesting willows, wheat, clematis, pea, radish and other plants were occasionally experimented with, several substances being used, as in other experiments. The development of the wings in these aphids could also be controlled.

IV. The Period during which the Development of the Wings may be Controlled.

Professor Clarke has suggested that the fate of the wing-buds may probably be determined near the end of the first molt, while Professor Woodworth thinks it can be predicted as early as one day after birth. The fact that one hundred per cent. of winged aphids can be produced on a twig charged with magnesium salts as late as three days after planting the twig, led Neiils to carry on a series of experiments along this line. By transplanting young aphids from a twig charged with distilled water to one charged with magnesium salts, he found that rose aphids could be made to produce wings if they were subjected to the magnesium solution within three days after birth. I have partly repeated his experiments and also extended them to other species with the following results:

It is seen from the above results that the period during which the development of the wing-buds can be controlled varies with the species. I have also observed in the course of my experiments that the aphids infesting the German ivy (*Senecio* sp.) develop the wing-buds within sixty hours after their birth.

TABLE III.

Date.	Name of Plant.	Total No. of Young Transferred.	Sol. on which Young were Born.	No. of Days left on H_2O.	Sol. to which they were Transferred.	No. that Remained Wingless.	No. that Developed Wings.	Per Cent. of Winged Forms.
Oct. 8–18, 1912	Rose	10	H_2O	1	$MgSO_4$	1	9	90
	"	10	"	2	"	0	10	100
	"	10	"	3	"	0	10	100
	"	10	"	4	"	2	8	80
	"	10	"	5	"	9	1	10
	"	10	"	6	"	9	1	10
Nov. 30–Dec. 9	"	10	"	1	"	1	9	90
	"	10	"	2	"	0	10	100
	"	10	"	3	"	0	10	100
	"	10	"	4	"	3	7	70
	"	10	"	5	"	8	2	20
	"	10	"	6	"	9	1	10
	Sonchus	10	"	1	"	0	10	100
	"	10	"	2	"	0	10	100
	"	10	"	3	"	0	10	100
	"	10	"	4	"	0	10	100
	"	10	"	5	"	0	10	100
	"	10	"	6	"	3	7	70
Feb. 10, 1913	Rose	10	"	1	"	0	10	100
	"	10	"	2	"	0	10	100
	"	10	"	3	"	1	9	90
	"	10	"	4	"	0	10	100
	"	10	"	5	"	2	8	80
	"	10	"	6	"	10	0	0
	"	10	"	8	"	9	1	10
	Sonchus	5	"	1	"			
	"	5	"	2	"			
	"	5	"	3	"			
	"	5	"	4	"	0	10	100
	"	5	"	5	"	0	10	100
	"	5	"	6	"	0	10	100
	"	5	"	8	"		2	20
	"	5	"	9	"		0	0
	Mustard	5	"	1	"			
	"	5	"	2	"			
	"	5	"	3	"			
	"	5	"	4	"		10	100
	"	5	"	5	"		10	100
	"	5	"	6	"		10	100
	"	5	"	8	"		8	80
	"	5	"	9	"		0	0

V. STRENGTH OF MAGNESIUM SULPHATE NEEDED TO PRODUCE THE WINGED APHIDS.

It must be conceded that no accurate methods of measuring the amount of any solution can be devised, for the reason that the solution must first pass through the plant tissue before it reaches the body of the insect. For the purpose of obtaining

some idea of the amount of the wing-developing substances to be effective in the development of the wing-buds, I instituted a series of experiments. In these experiments, the solutions of the magnesium sulphate, of various strengths, were poured directly into a series of tumblers, in each of which a rose twig, bearing a viviparous female, had been placed.

TABLE IV.

Date.	Solutions.	No. Winged.	No. Apterous.	Per Cent. Winged.
Oct. 9.....	*m*/2	8	2	80
	m/3	3	0	100
	m/4	5	0	100
	m/10	13	0	100
	m/20	33	0	100
	m/100	16	1	92
	m/200	112	4	95
Dec. 12...	*m*/20	6	1	86
	m/20	5	0	100
	m/80	18	1	90
	m/320	38	1	97
	m/50	10	0	100
	m/100	22	1	95
Feb. 25 ...	*m*/100	52	0	100
	m/200	52	0	100
	m/400 ?	12	2	90
	m/500 ?	212	4	98
	m/1000 ?	113	6	96

As will be seen from the table, I have been unable as yet to reach the lowest strength at which the majority of the aphids born on the twig would remain apterous. The table, however, suggests that this minimum amount may lie beyond the strength of an *m*/1000 solution.

It may also be added here that the susceptibility to the chemicals varies greatly with the species. *Euceraphis betulæ* on the birch tree growing on a sunny soil rarely or never produces apterous forms, while a considerable number of *Calaphis betulacolens* infesting the same host become winged. *Macrosiphum rosæ* also produce alate forms even on a relatively younger shoot, but it is utterly impossible to raise winged *Myzus persicæ* on a similar host without the application of a wing-developing substance.

VI. On the Physical Factors.

1. *Temperature.*—No special experiment for determining the effect of temperature upon the development of the wings has

been made. A part of my experiments, and also those of Professor Clarke, were carried on in our laboratory where a nearly uniform temperature was kept throughout a period of several days. A larger part of my work was done in an old greenhouse where the change of temperature between day and night was exceedingly great. I have also done a part of my work out of doors. In all of these cases, only a very few of the winged aphids appeared on the twigs charged with any of the non-wing-developing substances. Professor Clarke stated that he had subjected aphid-infested twigs charged with distilled water to a sudden change of temperature from 80° F. to 30° F., and had observed no effect on the wing development. These observations, therefore, establish the fact, I believe, that changes in temperature have no effect on the production of winged forms.

The popular conception that the approach of cold weather makes winged aphids appear may be explained in the following manner. Plants mature either at the time when the cold weather sets in or previous to that period. They also manufacture, through the agency of their leaves and under the influence of sunlight, starch, which at this maturing period is changed to sugar and stored away in other parts of the plant. This sugar is the most common wing-developing substance, as we have seen. Therefore, the aphids produce wings at the approach of cold weather, not because of the low temperature, but because of the presence of sugar in the plant tissues at that time.

2. *Wilting of the Plant.*—Professor Woodworth has pointed out that when the plants, especially cabbages on which he has made extensive observations, are badly infested with aphids and begin to wilt, there was a decrease in the birth rate of the insects and a "spontaneous appearance of the aphids with wing-pads was noticed." His explanation of this phenomenon is that the wilting of plants probably causes an effect similar to that produced by the magnesium salts, that is, it makes it possible for the wing-buds to have an even chance with the rest of the body to develop. Under more favorable conditions, he suggests that the growth of the wing-fundaments might be retarded on account of the faster growth of the other parts of the body. A morphological study bearing upon this point is pre-

sented farther on, but I shall now attack the problem by experimental means.

The weight of a number of fresh rose twigs, similar to those used in my other experiments, was recorded. One of these twigs was then planted in a tumbler containing distilled water, and one in a tumbler containing calcium chloride, in the manner already described. Three days later, the twigs were taken out of the tumblers, placed on a paper, and left to dry from four to twenty-four hours. The second or dried weight of each was recorded, and the twigs then replaced in their respective tumblers. Three to four apterous viviparous females were then transferred to each of them, and a careful watch kept for the young. The following tabulation shows the results obtained:

TABLE V.

THE EFFECTS FROM THE WILTING OF THE PLANT.

Date.	Chemicals.	Series.	Wt. of Fresh Plant.	Wt. of Dried Plant.	Per Cent. of Water Lost by Evaporation.	No. of Winged Aphids.	No. of Wingless Aphids.
Dec. 1...	H_2O	1	1.00 gr.	.90 gr.	10	1	24
	"	2	1.60 "	1.00 "	38	0	18
	"	3	1.60 "	1.40 "	12	0	4
	"	4	1.80 "	1.00 "	44	0	8
	"	5	2.00 "	1.50 "	25	0	12
	$CaCl_2$	6	1.60 "	.95 "	40	0	4
	"	7	.90 "	.50 "	44	0	16
	"	8	1.10 "	.65 "	40	0	6
	"	9	1.00 "	.50 "	50	0	4
	"	10	1.10 "	.60 "	45	0	8
Mch. 28..	H_2O	1	14.5 "	12.5 "	14	0	38
	"	2	1.60 "	.96 "	40	0	4
	$CaCl_2$	3	1.15 "	.60 "	49	1	8
	"	4	1.40 "	.90 "	36	0	4

Thus the mere wilting of the plant is not a factor in the production of the winged aphids. The drying out of the plant may, however, indirectly help to produce the winged insects by concentrating the ingredients in the plant tissues.

VII. MORPHOLOGICAL STUDY.

The young female aphids are, externally at least, well developed at the time of their birth. They have three pairs of legs and a pair of antennæ as stout as their mother's, but shorter. A pair of so-called honey-tubes and the caudal appendage are also

present. There is, however, no external indication of the wings or of their buds. The body is almost columnar, with a constriction back of the head, but with no marked differentiation between the thorax and abdomen.

In those individuals which ultimately become winged, the second and the third thoracic segments begin to swell laterally as well as dorso-ventrally. The swollen thorax is ordinarily noticeable at the end of the first ecdysis in the case of the rose aphids. Usually after the second, yet occasionally after the third ecdysis, in the case of the mustard aphis, a pair of hyaline wing-pads protrude from each side of the second and third thoracic segments. At the following ecdysis, the wing-pads become unfolded and the adult insect results. In those forms which remain apterous, no constriction between the thorax and abdomen occurs.

Other structural differences noticeable between the apterous and alate viviparous females of the common rose aphid, *Macrosiphum rosæ* (L.), may be tabulated as follows:

Winged Viviparous Female.	Wingless Viviparous Female.
Color—Dorsal marking present.	Dorsal marking absent.
Length—2.2 mm.	2.7 mm.
Antennæ—Articles I to VI dark throughout.	Only I, II, and VI dusky.
Sensoria on III—More than 60 found throughout the entire length.	About 12, all being found near the basal portion.
Color of Cornicle—Dark throughout.	Dark with lighter rings near the base and apex.

These are some of the more prominent differences. Minor differences, as, for example, the relative length, shape, size, etc., of the cornicle, cauda, antenna, etc., are numerous and cannot be enumerated here. It follows, then, that the factor which is responsible for the production of either the apterous or the alate forms not only determines the fate of the wings, but also causes other morphological changes as above mentioned.

Although no special effort was made to study the histological differences between the two forms, the sectioned material showed that there is a less amount of muscles and ectodermal cells, but more of fat body in the apterous form as compared with the alate individual.

VIII. General Discussion.

As the result of our experiments, we have been able to distinguish two groups of substances, one that produces and the other that does not produce winged aphids. *Clarke* (1901), *Neiils* (1912) and *Woodworth* (1908) held that the presence of magnesium in the plant tissue might be responsible for the phenomenon under discussion. My experiments have shown that the magnesium salts are not the sole substances that produce the winged aphids. Other substances, namely, the salts of heavy metals, had a similar effect on the development of wings. Consequently, it becomes clear that what is really responsible for the appearance of the winged aphids is the excess of magnesium and other wing-producing substances over the non-wing-producing substances contained in the plant sap.

Morgan (1909) presented an analysis of the leaves of plants, the rose and the maple, at two different seasons of the year, namely, June and October. His table showed, as we should expect, a marked difference in the amount of ash contained in the leaves of the two kinds of plants at the same time of the year. A similar difference was also pointed out in the case of the same plant at two different seasons of the year. The chemical analysis of plants is, then, in accordance with the general observation in the field that the alate forms are more abundant during the autumn than in the spring and that the winged forms of the same species are more abundant on one kind of plant than on another even at the same period of the year.

Morgan's table is also valuable in estimating the proximate amount of wing-developing substances that may be effective. According to the analysis, the percentages of ash contained in the fresh rose leaves in October and June are 3.61 and 3.69, respectively. The difference between these two figures, or 0.541 per cent., may then be regarded as the maximum amount of the wing-developing substances which would be effective when added to the spring leaves.

I have already given an explanation of why the drying of plants produced alate forms as reported by Woodworth (1908). However, I did not discuss the secondary effect of temperature on the development of the wing. Recently Ewing (1916) stated

that a great number of alate forms would appear under a certain optimum temperature in the form he had been experimenting with, namely, *Aphis avenæ*. I have already shown that the temperature had nothing to do with causing the appearance of the winged forms under a well-controlled environment. Ewing's result, like that of Woodworth on the effect of dryness, was obtained under natural conditions, with perhaps little attention paid to the chemical constituents of the host plant. It is strongly doubted whether any winged forms would have appeared, had they been reared under his optimum temperature but on the host charged with any one of the non-wing-developing substances. In fact, I believe that the appearance of winged forms under his "optimum temperature" is due, not so much to the direct action of heat as such, but indirectly to either an increase in the solubility of the wing-developing substances or to a decrease of the non-wing-developing substances.

Tannreuther (1907), who has made extensive observations on the life history as well as the genetic cycle of *Pterocomma salicis* and *P. salicicola*, says: "The prevalent idea that the development of aphis is unstable and controlled directly by external conditions is certainly very misleading especially in the idea that unfavorable conditions or lack of food is a direct cause for the appearance of the winged and sexual forms. We find in the species studied that just the reverse is true, and that the greatest number of winged forms are found in the second parthenogenetic generation; here in some instances ninety-five per cent. may become winged, especially those found on the rose in good condition which furnished an abundance of food." *Pterocomma bicolor* and *P. rufurum* found in the vicinity of the University of California campus, however, reacted to the chemicals, but they were peculiar in that they did not produce sexual forms at the sixth generation.

Now I come to the consideration of the question which was set forth by Tannreuther (1907), to wit, "Why some of the hypodermic cells of the thorax begin to divide, emarginate and form the adult wings in a few days." According to Anderson (1893) and others there are two groups of salts, one that excites the muscles and one that has little effect upon them. Our so-called

wing-developing substances correspond with the former, and the non-wing-developing substances to the latter. Further, so far as these non-wing-developing substances are concerned, Herbst (1906), Loeb (1907), and others have proven them to be injurious to other classes of animals. For example, Herbst (1906) found that the addition of potassium salts to sea water suppressed the development of the arms of the *Echinus* larvæ. These investigators have also found that the development of the alimentary canal of the *Echinus* larva was impossible in magnesium-free sea water. Again, Fischer (1903) and others have proven that magnesium salts counteract the effect of saline purgative. All these results obtained with other animals by various investigators suggest that wing-developing and non-wing-developing substances exert, respectively, characteristic action on the organic matter. We have at least two evidences that tend to show that in all probability the two phenomena of the appearance of the winged or wingless individuals may be due to the physiological actions osmosis and surface tension. One of these is the presence of more of the fat cells, which are derived from mesoderm cells, in the apterous forms than in the alate forms, and the other is the fact that the cells of the apterous forms are slightly larger than those of the alate forms. The first of these evidences suggests the probable degeneration of muscle and probably nerve cells at the time when the wing cells are in the process of differentiation. The degeneration may, it must be said, be due to the increase in the cytoplasmic area of the cell and the subsequent loss of the coördination between the nucleus and its surrounding cytoplasm, as is manifested in the case of the fat body. The difference in the size of the two forms may not only strengthen the view just presented, but also explains why the winged forms resemble the male, and the apterous, the sexual females; for the two winged forms, the male and the alate viviparous females are much more highly developed than the two wingless sisters, and are produced by the same factor—the decrease in surface tension and osmosis. These physiological factors osmosis and surface tension produce the male if they act on the egg at the time of polar-body formation, and the winged viviparous female if they act on the larva within three days after birth.

In concluding this paper, it may be added here that in the year 1909 von Baehr made the following prediction: "Meine Beobachtungen an *Schizoneura lanigera* führen mich zu dem Schluss, dass der Factor, welcher bei den Embryonen die zu Geschlechtstieren werden, den Impuls zur Ausbildung der Winterovarien und zur Degeneration der schon ziemlich entwickelten parthenogenetische Eiröhren gibt, wahrscheinlich im kausalen Zusammenhang mit der Entwicklung der Flügel, Brustmuskalatur, Sinnesorgane u. s. w. des Muttertieres steht in dem sie sich entwickeln. Derselbe Factor dürfte wohl die Veranlassung sein, dass einige Eier sich zu Männchen entwickeln, wie es im speziellen Teil ausgeführt wurde, werden nämlich fast immer ausser geschlechtlichen Weibchen noch 1–4 Männchen von derselben Mutter geboren."

IX. Aphis Species Mentioned.

Only the common or vernacular names have been given so far in this paper. It, however, seems necessary to add the scientific names of the aphids I have made use of for the reason that the species of aphids infesting the same host frequently vary with the locality as well as with the season of the year. A list of the specific names, together with some of the more common synonyms, is given below:

1. The small birch aphis:
   Caraphis butulaecolens Fitch.
   Siphonocallis butulecolens Fitch.
2. The clematis aphis:
   Myzus persicæ Sulz.
   Aphis persicæ.
   A. dianthi.
   Rhopalosiphum dianthi.
   Siphonophora achyrantes.
   Myzus malvæ.
   Rhonpalosiphum persicæ Sulz.
3. The German ivy aphids:
   Aphis senecio Swaine (MS.).
4. The California maple aphids:
   Thomasia californica Shinji.

5. The mustard aphis:
   Aphis brassicæ Linn.
6. The Periwinkle aphis:
   Myzus circumflexivum.
7. The green pea aphis:
   Macrosiphum ulmariæ (Schv.).
   Aphis ulmariæ Schr.
   A. pisi Kalt.
   Siphonophora pisi Koch.
   S. ulmariæ Kalt.
   Nectraophora pisi Oestl.
   N. destructor Johns.
8. The large rose aphis:
   Macrosiphum rosæ Beaum.
   Aphis rosæ Linn.
   A. dipsaci Schr.
   Siphonophora rosæ Beaum.
   Nectarophora rosæ Linn.
9. The large sonchus aphis:
   Macrosiphum solanifoliæ.
10. The small sonchus aphis:
    Aphis gossipii Glov.
    Aphis citrulli Ashm.
    A. cucumeris Forbes.

X. Summary.

Results of the observations and experiments reported above may briefly be summarized as follows:

1. Either an apterous or an alate parthenogenetic female may bear young larvæ, some of which may finally attain the winged condition, while the others may remain wingless throughout life.

2. When newly born aphids were reared on rose twigs planted in tumblers, containing washed and sterilized sand which had previously been saturated with the solution of a certain substance, nearly one hundred per cent. of winged individuals appeared on the twigs, while either none or few winged forms developed on the twigs charged with any one of another set of chemi-

cals. For the sake of making a distinction between these two groups of substances, we may call the former the "wing-developing" substances, and the latter, the "non-wing-developing" substances.

3. As far as the tests go, the salts of the alkalis (Na, Cl, K, etc.) and alkaline earths (Ca, Br) with the exception of magnesium, distilled water, urea, alum and others were shown to belong to the non-wing-developing substances, while the salts of the heavy metals, and of magnesium, sugar, and perhaps some others belong to the category of wing-developing substances.

4. The wing-developing substances were only effective when applied within a certain period after birth. This period varied with temperature and also with the species, *e. g.*, the maximum time for the rose aphis, *Macrosiphum rosæ* L., during the early summer was found to be from 2 to 3 days, while under the same conditions from 5 to 7 days was the maximum in the case of the sonchus aphids, *Macrosiphum solanifoliæ* Ashm., the mustard aphis, *Aphis brassicæ* L., etc.

5. The amount of magnesium salts and also of other wing-developing substances needed to produce winged aphids was very small. Subjection to a *m*/100 solution of magnesium sulphate for 12–24 hours produced nearly one hundred per cent. of winged aphids, so far as *Macrosiphum rosæ* was concerned.

6. When twigs planted in the sand saturated with distilled water and calcium chloride were subjected to a drying process, the young aphids born on these twigs remained apterous, whereas on those that had been charged with solutions of magnesium salts or some other wing-developing substances, the greatest number of the winged aphids appeared.

7. When newly born aphids are subjected to a mixed solution of wing-developing and non-wing-developing substances, winged aphids may or may not appear, according to whether or not the solution contains an excess of the wing-developing over the non-wing-developing substances.

8. Variation in temperature, or a sudden change from as high as 100° F. to as low as 35° F., failed to produce winged aphids on the twigs charged with non-wing-developing substances.

LITERATURE CITED.

Anderson, Stuart

'90 Nickel and cobalt: their physiological action on the organism. Journ. of Anat. and Physiol., Vol. XVII.

von Baehr, W. S.

'09 Die Oögenese bei einigen viviparen Aphiden usw. Arch. Zellf., Vol. III.

Clarke, W. T.

'03 Wing development in plant lice. Journ. of Tech. U. C. Student Publ., Vol. I., No. 3.

Ewing, H. E.

'16 Eighty-seven generations in a parthenogenetic pure line of *Aphis avenæ* Fabr. BIOL. BULL., Vol. XXXI., No. 2.

Fischer, M. H.

'00 On the production and suppression of glycosuria in rabbits through electrolytes. U. C. Publ. Physiol., Vol., I., No. 9.

Herbst, C.

'93 Experimentalle Untersuchungen über den Einfluss der veränderten chemischen Zusammensetzung des umgebenden Mediums auf die Entwickelung der Tiere: I. Versuche an Seeigeleiern. Zeitsch. Wissensch. Zoöl., Vol. LV.

Loeb, J.

'08 Über den Einfluss von Alkalien und Säuren auf die Embryonale Entwickelung und das Waschstum. Arch. Entwicklungsmec., Vol. VII.

Morgan, T. H.

'09 A biological and cytological study of sex determination in Phylloxera and aphids. Journ. Exper. Zoöl., Vol. VII.

Neiils, J. D.

'12 Wing production in aphids. Entom. News, Vol. XXIII.

Slingerland, M. V.

'93 Notes from Cornell Insectory, II. Some observations upon plant lice, Vol. XXI.

Tannreuther, Geo. W.

'07 History of germ cells and early embryology of certain aphids. Zoöl. Jahrb. Abt. Anat. u. Ont., Vol. XXIV.

Woodworth, C. W.

'08 Winged aphids. Entomol. News, Vol. XIX.

EYE-SPOTS IN DIGENEA.[1]

ERNEST CARROLL FAUST, PH.D.

INTRODUCTION.

While considerable work has been done on the eye structure of the free-living Plathelminthes and ectoparasitic Trematoda, no adequate study of the eye-spots of the digenetic Trematoda has been made. In fact, although the nervous system of this group has been comparatively well known since the days of Lang (1880), students of Trematoda have been content with the mere mention of the occurrence of eye-spots in this or that species, or at most with a description of an isolated case and perhaps a reference to this organ in Turbellaria or ectoparasitic Trematoda.

There remain unanswered, then, two fundamental questions relative to the eyes of Digenea, (1) What is the fundamental structure of this organ, and (2) how extensive is the development of the organ in the group.

HISTORICAL.

The earliest mention of eye-structure in Digenea was made by O. F. Müller (1773: 68, 70) for *Cercaria lemna*. He suggested that the two black pigment spots of these larvæ were probably eyes. Mehlis (1831) described for the first time pigment eyes in miracidia (*Cathemasia hians*). In 1832 von Nordmann (II., 140) made record of the eye-spots in the miracidia of *Bunodera luciopercæ*. Shortly afterward the X-type of pigment pattern in the embryos of *Fasciola hepatica* and the two beehive spots in the embryos of *Cyclocœlum mutabile* were described. Meanwhile other records of cercariæ with eye-spots had been published. O. F. Müller in 1776 (*C. inquieta*), Hemprich und Ehrenberg in 1831 (*C. alata*), J. Müller in 1850 (*C. setifera* and *C. elegans*), and La Valette St. George in 1855 (*C. ocellata*) for cercariæ, and de Filippi in 1857 for the amphistomulum of

[1] Contributions from the Zoölogical Laboratory of the University of Illinois no. 114.

Diplodiscus subclavatus (Goeze), together with an occasional record of the pigment-spots in miracidia—these observers showed the rather wide distribution of this type of sense organ in the group.

In his second memoir de Filippi (1857: 433) described the pigment spots of *Cercaria echinocerca*. Among the granules, he wrote, were two large pockets of irregular form but symmetrical arrangement on the sides of the esophagus, comparable to the eyes of the amphistome, while within the pigment pocket was a nucleus which represented a veritable rudimentary lens. Leuckart (1863: 465) expressed his credulity about the true lenticular function of the nucleus, but saw the relationship to the eye-spots of ectoparasitic trematodes. He mentioned the occasional presence of such organs in the distome, even in the definitive host. Later (1886: 26) he emphasized their homology to the eyes of the Monogenea.

Accounts of the eye structure in ectoparasitic trematodes have been given by Lang (1880), Goto (1894), Hesse (1897), Beers (1901), and André (1910). Lang worked out the histology of this organ carefully for *Tristomum coccineum*. He found the roots to be a branch of the weak posterior dorsalis, the eye itself to consist of (1) a saucer-shaped pigment cap, (2) a spherical or oval lens, (3) a retinal ganglion cell, and (4) muscle cells. Goto (p. 81) was unable to believe that the eyes of his species were functional because (1) of the position of the pigment granules between the source of light and the lens and (2) because of the degeneracy in part of the lens itself. He was inclined to consider them as temperature sense organs.

In very young cercariæ of *Diplodiscus subclavatus* Looss (1892: 165) found two or three limpid cells with refractive nuclei situated on each side of the esophagus. He thought these to be connected with the developing eye-spots, "die oberste vorzugsweise den lichtbrechenden, die unteren vielleicht die percipirenden Apparate darstellen. Alle drei bleiben aber noch einfache Zellen." Osborn (1903), in connection with the histology of *Cotylaspis insignis*, described eyes for the adults of this species as "located at the front of the pharynx between it and the posterior nerve trunk. . . in close contact with the latter." Each

eye at its best consisted of a pigment cup in which the contents are figured but not recognized as lenticular in function. Monticelli's study (1914) of the eye structure in *Cercaria echinocerca* Fil. is not as clear as the work of the earlier investigator on this species. The eyes in *Cercaria equitator* (Ssinitzin, 1911: Fig. 54) are of interest because they correspond to the X-type originally described for the miracidium of *Fasciola hepatica*. Cort (1915) discussed the eye-spots in monostome and amphistome cercariæ but failed to show their connection with the brain.

Morphology.

The writer has studied intimately the eyes of several monostome and distome cercariæ (Faust, 1917, 1918, 1918*a*). Of twenty-eight species of cercariæ which have come under the writer's observation, seven possess pigmented eyes and four have non-pigmented eyes. The existence of these non-pigmented eyes was first described by the writer for *Cercaria racemosa* and *C. gracillima* (1917).

The eye-spots of the binoculate species are usually in direct connection with the posterior dorsal nerve trunks (Faust, 1918). However, the forked-tailed species, *Cercaria gigas* (Faust, 1918*a*) has connections with the anterior dorsal rami. While the posterior dorsal trunks are, on the whole, much smaller and less important than the corresponding ventral trunks, the ramus to the lateral eye on each side is conspicuous. The cyclopean eye in the trioculate species (confined to certain monostome cercariæ) arises from a fused anterior dorsal nerve trunk. It receives a blunt nerve fiber from below. Thus it differs markedly from the anterior pair of eye-spots in the Monogenea which are innervated by a branch of the same nerve which innervates the posterior pair.

Description of the eye-spot.—In the pigmented eye-spots the pigment consists of a cluster of rather large dark-brown granules, grouped together so as to form a deep cup. At the base of the cup the granules lie several layers deep, but toward the mouth they are seldom more than one layer deep. The opening of the cup in the case of the paired eyes is usually dorsolaterad, whereas the cyclopean eye opens dorsomesad (Fig. 1). Within the pigment

cup is a spherical body, barely touching the pigment granules, opalescent, and containing within it an oval nucleus. This spherical body is the enlarged nerve-cell ending with its nuclues. The nerve may be clearly traced out of the mouth of the pigment cup where it merges into the dorsal branch of the nerve trunk

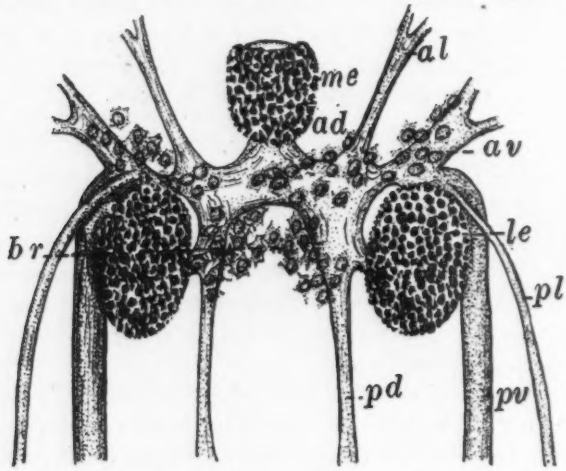


FIG. 1. Dorsal view of the central nervous system of *Cercaria pellucida* Faust, showing paired lateral and cyclopean eye-spots and their connection with the dorsal nerve trunks. *ad*, anterior dorsalis; *al*, anterior lateralis; *av*, anterior ventralis; *br*, ganglion cells; *le*, lateral eye; *me*, cyclopean eye; *pd*, posterior dorsalis; *pl*, posterior lateralis; *pv*, posterior ventralis. × 660.

(Fig. 2). Thus the end organ of this nerve is imbedded in the center of the pigment cup. In the mature cercaria the eye-spot is sunken beneath the basement membrane.

The above description holds likewise for the pigmentless eye-spot, except that in this type of organ no pigment is present. It is recognized in the living cercaria as a round milky body slightly whiter and less transparent than the surrounding parenchyma cells. In stained material its nucleus is conspicuous and its connection with the main nerve center can be made out. In *Cercaria pellucida* although there are ganglion cells scattered superficially along the main nerve trunks no cell is found at the base of the cup. The pigment cup varies in shape from

saucer-shape to deeply pocketed (*C. gigas*). In all cases recorded for the Digenea the opening of the cup is toward the source of light. Thus the organ may function as a light-percipient mechanism, although its function is undoubtedly impaired by the position

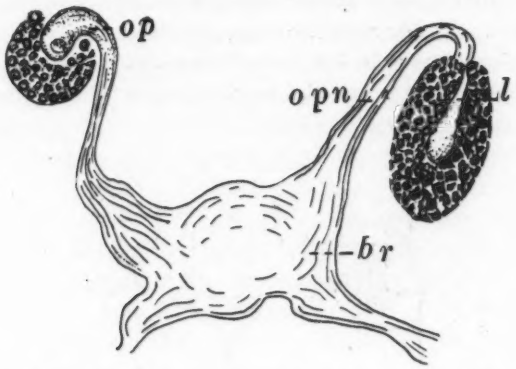


FIG. 2. Section through young *Cercaria pellucida* showing relation of pigment cup to nerve ending in the paired lateral eyes. *br*, brain center; *l*, "lens" of authors; *op*, optic cell; *opn*, optic nerve. × 660.

of the nerve fiber in front of the "lens." This type of eye-spot shows no differentiation into rod and cone structure, such as André (1910) has depicted for *Polystomum integerrimum*. The mechanism at its best is decidedly an inferior one.

DEVELOPMENT.

The pigment eye-spot of the Digenea, as studied by the writer in *Cercaria pellucida* Faust, *C. urbanensis* Cort and *C. gigas* Faust, presents significant points in development. At an early stage of the germ ball it becomes differentiated in accordance with the precocious development of the nervous system in the group. A branch of the posterior dorsalis, with a single nucleus, pushes out from the nerve center to the dorsolateral margin of the embryo. As it reaches a position near the surface somewhat posterior to the cerebral mass, the ectodermal layer of the embryo pushes inward, just posterior to the nerve, so that a pocket is formed with the opening opposed to the nerve cell. The nerve fiber and enlarged end then twist around the inner wall of the

pocket so that the bulbous end with its nucleus comes to lie within the cup (Fig. 3). Although the ectodermal cells of this impocketing are at first visible, they appear to play no further part in the development of the organ. Pigment granules are not present until the nerve ending comes to occupy a position within the pocket. Shortly afterward these granules, at first a golden-brown color, come to be laid down between the nerve ending and the ectodermal cup. As the organ matures the cell with its

FIG. 3. Section through young germ ball of *Cercaria urbanensis* Cort, showing formation of pigment granules between optic cup and nerve cell. *op*, optic cell; *opc*, optic cell nucleus; *opn*, optic nerve. × 1,460.

nucleus enlarges and assumes the appearance of a lens, although it does not take on concentric rings. The pigment granules become more numerous and darker in color so that *en masse* they look black. Meanwhile the ectodermal pocket becomes less and less conspicuous so that in later embryos it entirely disappears.

While the writer can agree with Looss (1892: 165) that a nucleus is concerned with the formation of the "lens," this nucleus is derived from the nerve center rather than from indifferentiated cells *in situ*. Moreover, the pigment accumulation is not brought about by a secondary nucleus but is the direct result of the apposition of the end organ and the optic cup. Its shape depends on the shape of the optic cup. The melanoidin precipitation is probably a by-product of the nervous system, as has been shown for *Cercaria pellucida*.

The eye-spots are, then, specialized end organs around which a considerable amount of pigment accumulates on account of

increased activity. The pigment is possibly both light- and heat-percipient.

Distribution of Pigment in the Digenea.

For convenience the pigment spots in miracidia, cercariæ, immature and mature trematodes will be discussed separately. Leuckart (1886: 73) states that in general eyes are lacking in miracidia, while Ward (1918) defines a miracidium as possessing an eye-spot. In treating the nervous system of Digenea, Braun (1892) remarks that pigment eyes in adults are exceptional, citing *Acanthopsolus ocellatus* (Levinsen) as the only adult in this order in which eye-spots are known. A survey of the group shows that the data are isolated and in many cases incomplete.

The common examples usually cited in miracidia are the bee-hive pigment eyes of *Cyclocœlum mutabile* and the X-type of *Fasciola hepatica*. Among monostomes it is the common thing to find pigment flecks in the ciliated larva, even in the eggs *in utero*. Among distomes the only species found with pigmented eyes in miracidia are *Fasciola hepatica*, *F. hepatica* var. *ægyptiaca*, *Bunodera luciopercæ*, and *Cathemasia hians* (fide Leuckart). The miracidium of the holostome, *Strigea cornucopiæ* (von Linstow, 1885) possesses conspicuous eye-spots. No record of pigment spots in the miracidia of amphistomes has been found, although Looss (1892, 1896) has worked out the details in the early stages of the life history of four representative species. The miracidia of the Aspidocotylea are not well known.

No pigment eye-spots have been described for the parthenitæ.

Turning to the cercariæ, one is struck by the disproportionally wide distribution of pigmented eyes. In monostomes the cercariæ thus far described possess eye-spots of the binoculate or trioculate types (Fig. 1). Amphistome cercariæ are commonly binoculate. Both monostome and amphistome cercariæ have a considerable amount of pigmentation over the nerve center around the eyes. However, the greatest number of records of eyes in cercariæ concerns the distomes. They are recorded for gymnocephalous species by Ercolani (1881: 54, 55), and Looss (1896: 204–210); for setiferous species by La Valette (1855), Odhner (1914) and de Filippi (1857: 433); for stylet cercariæ by Ssinitzin

(1911) and Faust (1917); for furcocercous forms by La Valette (1855), Sonsino (1897), Cort (1914, 1917), O'Roke (1917) and Faust (1917, 1918*a*); and, in addition, for many other less closely related species by O. F. Müller (1773, 1776), Hemprich und Ehrenberg (1831) and Ssinitzin (1911). Holostome cercariæ have not been shown to possess pigment eye-spots. The existence of the cercaria stage in Aspidocotylea is still uncertain.

After the cercaria stage the pigment may persist for a little while but is usually lost on the arrival of the fluke within the definitive host. However, the pigment granules persist in some cases, being found in the mature worm. Although the pigmentation of the monostome cercaria is carried over to the encysted monostomulum, pigmentation in adult monostomes is quite unknown. On the other hand, several amphistomula which have reached their final host still have a clump of pigment granules around the optic end organ. Among distomes the distomula and adults known to possess pigment eye-spots belong to the Allocreadiidæ or closely related species. In many cases the pigmentation is faint and the "lens" degenerate. No holostome adults are recorded as having pigmented eyes. Whereas the other stages in the life cycle of the Aspidocotylea are in doubt, the group is represented in the adult stage by one pigmented species, *Cotylaspis insignis*.

Reviewing the entire field of the Digenea, pigment eye-spots are found in representative species of all suborders of the Prostomata at one time or another in the life cycle of the individual. Among the monostomes, both miracidia and cercariæ commonly possess these organs. Amphistome miracidia usually lack pigment eyes, while amphistome cercariæ are usually binoculate. Holostome miracidia are pigmented (at least in one species), while neither cercariæ nor adults are pigmented. Few distome miracidia are marked by pigment eye-spots, although a pigmented optic organ is common in cercariæ of various distome families and usually present in Allocreadiide distomula and adults. The only recorded instance of pigmented eye-spots in miracidia, cercariæ, and adults is that of *Bunodera luciopercæ*.

TABLE SHOWING PIGMENTED EYE-SPOTS IN DIGENEA.

Group.	Miracidium.	Cercaria.	Adult.
Gasterostomata.....	none	none	none
Prostomata			
Aspidocotylea.....	?	?	present in *Cotylaspis insignis*
Monostomata.....	common	common	none
Amphistomata....	none (?)	common	in amphistomula and some adults
Distomata.....	seldom	common	in Allocreadiide species
Holostomata.....	in *Strigea cornucopiae*	none (?)	none (?)

GENERAL CONSIDERATIONS.

The occurrence of eye-spots in various stages of the life cycle of Digenea and in representative species of all suborders of the Prostomata at one time or another in the life cycle show the fundamental significance of the eye structure. The preservation of the pigmentation and of the "lens" in this or that group has been decidedly erratic and apparently unrelated to the systematology of the group. The great trend has been one of degeneration. If the sporocyst or redia ever possessed eye-spots they have lost all vestiges of them. The absence of eye-spots in many cercaria-trematode generations shuts out any clue of pigmentation at a former period. In some cases absolutely no evidence exists in the life cycle of a possible former pigmentation. In spite of these facts, the widespread pigmentation throughout the Digenea warrants the belief that pigmentation was general throughout the Class at some former time.

In most cases pigment eye-spots have been described because they were conspicuous. It is highly probable that in many species pigmentless eye-spots exist. The three cases on record (*Cercaria racemosa*, *C. gracillima* and *C. minor*) were found only by a most careful study of the worms under high powers of the microscope. While they are undoubtedly functionless, a record of their existence extends the knowledge of the extent of eye-spots and of the types of degeneration that have occurred under conditions of endoparasitism.

IMPORTANT REFERENCES.

André, J.

'10 Zur Morphologie des Nervensystems von Polystomum integerrimum Froel. Zeit. wiss. Zool., 95: 191–220, 13 figs.

Beer, Th.

'01 Über primitive Seh-Organe. Wien. klin. Wochensch., 14: 314–324.

Braun, M.

'92 Bronn's Tierreich. Vermes. Trematodes. Leipzig. 619 pp., 27 pl.

Cort, W. W.

'14 Larval Trematodes from North American Fresh-Water Snails. Jour. Parasit., 1: 64–84, 13 figs.

'15 Some North American Larval Trematodes. Ill. Biol. Monogr., 1: 447–532, 8 pl.

'17 Homologies of the Excretory System of the Forked-Tail Cercariæ. Jour. Parasit., 4: 49–57, 2 figs.

de Filippi, Ph.

'57 Deuxième Mémoire pour servir à l'histoire génétique des Trématodes. Mem. r. Acc. Sci. Torino, (2) 16: 419–442, 2 pl.

Ercolani, G.

'81 Dell' adattemento della specie all' ambiente. Nuove ricerche sulla storia genetica dei Trematodi. I. Mem. r. Acc. Ist. Bologna, (4) 3: 46–111; 3 Tav.

Faust, E. C.

'17 Notes on the Cercariæ of the Bitter Root Valley, Montana. Jour. Parasit., 3: 105–123, 1 pl.

'18 Life History Studies on Montana Trematodes. Ill. Biol. Monogr., 4: 1–120, 9 pl.

'18a Studies on Illinois Cercariæ. Jour. Parasit., 4: 93–110, 2 pl.

Goto, S.

'94 Studies on the Ectoparasitic Trematodes of Japan. Jour. Coll. Sci. Imp. Univ. Japan, 8 (1): 1–273, 27 pl.

Hemprich und Ehrenberg.

'31 Symbolæ physicæ. Berol. (Cited after Braun.)

Hesse, R.

'97 Untersuchungen über die Organe der Lichtempfindung bei niederen Thieren. II. Die Augen der Plathelminthen. Zeit. wiss. Zool., 62: 527–582, 2 Taf.

Lang, A.

'80 Untersuchungen zur vergleichenden Anatomie und Histologie des Nervensystems der Platyhelminthen. II. Uebèr das Nervensystems der Trematoden. Mitth. Zool. Sta. Neapel, 2: 28–56, 3 Taf.

La Valette St. George, A.

'55 Symbolæ ad Trematodum Evolutionis Historiam. Berol. 38 pp. 2 Tab.

Leuckart, R.

'63 Die Menschlichen Parasiten. Leipzig u. Heidelberg. 766 pp.

'86 Die Parasiten des Menschen. Leipzig. 897 pp.

Linstow, O. von.

'85 Beobachtungen an bekannten und neuen Nematoden und Trematoden. Arch. Naturgesch., 51: 235–255, 3 Taf.

Looss, A.

'92 Ueber Amphistomum subclavatum Rud. Leuckart's Festschrift, Leipzig, 147–167, 2 Taf.

'96 Recherches sur la Faune parasitaire de l'Égypte. Prem. Partie. Le Caire. Mem. l'institute Egyptien, 3: 1–250, 17 pl.

Mehlis, E.

'31 Anzeige von Creplin's Novæ Observationes de Entozois. Isis, 1831, pp. 166–199.

Monticelli, Fr. Sav.

'14 Ricerche sulla *Cercaria setifera* di Joh. Müller. Ann. Mus. Univ. Napoli, 4, no. 5. 49 pp., 5 pl.

Müller, J.

'50 Ueber eine eigenthümliche Würmlarve aus der Classe der Turbellarien und aus der Familie der Planarien. Arch. Anat. Physiol., 1850: 485–500, 2 Taf.

Müller, O. F.

'73 Vermium Terrestrium et Fuviatilium Historia. Havniæ et Lipsiæ. 135 pp.

'76 Zoologiæ Danicæ Prodromus. Havniæ.

Nordmann, A. von.

'32 Mikrographische Beiträge zur Kenntniss der Wirbellosen Thiere, II. Berlin. 150 pp., 10 pl.

Odhner, Th.

'14 Cercaria setifera-von Monticelli- die Larvenform von *Lepocreadium album* Stoss. Zool. Bid. Uppsala, 3: 247–255, 1 Taf.

O'Roke, E. C.

'17 Larval Trematodes from Kansas Fresh-Water Snails. Kan. Univ. Sci. Bull., 10: 161–180, 7 pl.

Osborn, H. L.

'03 On the Habits and Structure of Cotylaspis insignis Leidy, from Lake Chautauqua, New York. Jour. Morph., 18: 1–44, 2 pl.

Sonsino, P.

'97 Cenni sulle forme larvali di Trematodi osservate nei Gasteropodi di acqua dolce dei diu torni di Pisa. Atti Soc. tosc. Nat. Proc.-verb., 10: 249–253.

Ssinitzin, D. Th.

'11 La génération parthénogénétique des Trématodes et sa descendence dans les mollusques de la Mer Noire. Mém. Acad. Sci. St. Petersbourg, (8) 30, no. 5. 127 pp., 6 pl.

Ward, H. B.

'18 Fresh-Water Biology. Parasitic Flat Worms. New York. 98 pp.